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THE ANTIGENIC PROPERTY OF POLLENS*

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Since Wolff-Eisner [1] suggested in 1906 that the symptoms of pollen disease resemble the phenomena of experimental anaphylaxis, the relationship of these conditions has been extensively studied clinically and experimentally. Soon after 1906 Billard and Maltet,[2] Meltzer [3] and Billard [4] suggested that there were certain clinical and laboratory similarities of these conditions, while many others submitted experimental evidence to support this view. Dunbar,[5] Koessler,[6] Heyl,[7] Alexander,[8] Harrison and Armstrong [9] and Parker [10] reported the demonstration of severe or fatal anaphylactic symptoms following the injection of pollen extracts into specifically sensitized guinea-pigs. Parker,[11] using the uterine strip method, demonstrated that active sensitization of guinea-pigs with pollen extracts can be accomplished, an observation that was to be anticipated considering the protein content of pollens. Dunbar, Koessler and Alexander reported successful passive sensitization of normal guinea-pigs by injecting into them the blood serum of patients suffering from pollen disease or of guinea-pigs sensitized with pollen extracts. There also are numerous clinical reports

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* A preliminary report of this paper was given at the meeting of the American Association for the Study of Allergy, Chicago, 1924.

1. Wolff-Eisner: Das Heufieber, München, 1906.
2. Billard G., and Maltet L.: J. de physiol. et de path. gén., 1907, p. 304.
3. Meltzer, S. J.: J. A. M. A. **55**:1021 (Sept. 17) 1910.
4. Billard, G.: Lancet, 1910, pp. 179, 1208.
5. Dunbar, W. P.: Deutsch. méd. Wchnschr. **37**:578, 1911.
6. Koessler, K. K.: Forchheimer's Therapeusis of Internal Diseases **5**:6, 1914.
7. Heyl, F. W.: Tr. Am. Chem. Soc. **39**:1470, 1917.
8. Alexander, M. E.: J. Immunol. **8**:457 (Nov.) 1923.
9. Harrison, W. T., and Armstrong, C.: Pub. Health Rep. **39**:1261 (May 30) 1924.
10. Parker, Julia T.: Proc. Soc. Exper. Biol. & Med. **18**:237, 1920-1921; J. Immunol. **9**:515 (Nov.) 1924.
11. Parker (Footnote 10, first reference).

of more or less severe anaphylactic-like symptoms in man following the prophylactic use of small doses of pollen preparations.

On the other hand, Cooke, Flood and Coca [12] in well controlled experiments were not able to demonstrate definite anaphylactic phenomena, passive sensitization or the production of specific antibodies. Because of this failure to show the antigenic property of pollen extracts, Cooke and his associates and Coca have attempted to rule pollen disease out of the category of anaphylactic phenomena. Coca [13] divided all cases of hypersensitiveness into two groups, the anaphylactic and the allergic, and places pollen disease under the allergic group.

Zinsser [14] recently stated his view of the question as follows: "While therefore we must again call attention to the important difference between true anaphylaxis and hay-fever because of the probable rôle of inheritance, we can also emphasize the many points of definite analogy between the two conditions."

In earlier studies of pollen disease we had observed that the sensitization of guinea-pigs with pollen extracts was not easily accomplished. Because of this observation and because of the conflicting reports in the literature we planned a series of experiments to study the antigenic properties of pollen extracts. Our methods are given as developed.

PREPARATION OF POLLEN EXTRACTS

The pollen extracts were all prepared in the same manner except for variations in sodium chlorid content and in ratio of pollen to extracting solution. The dried pollens were weighed and placed in sterile thick walled bottles, which contained small irregular glass beads. These bottles were then shaken in a shaking machine until the pollen grains were fairly completely macerated, as determined microscopically. The macerated pollen was then extracted at room temperature in the shaking machine with a chloroform saturated 0.85 per cent. sodium chlorid solution [15] that contained 0.25 per cent. sodium bicarbonate solution. The ratio of dry pollen to extracting solution varied from 1 : 10 to 1 : 30 depending on experimental needs.

After from twenty-four to thirty hours' extraction, the solutions were allowed to settle, centrifugated at high speed, decanted, filtered through Berkefeld filters, and after sterility had been proved anaerobically and aerobically were sealed in glass and kept in an icebox until used. The nitrogen content of all extracts was determined by the Kjeldahl method.

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12. Cooke, R. A.; Flood, E. P., and Coca, A. F.: J. Immunol. **2**:217 (Feb.) 1917.

13. Coca, A. F.: Tice Practice of Medicine **1**:179, 1920.

14. Zinsser, H.: Infection and Resistance, Ed. 3, pp. 487-489.

15. Extract *A* used in the first experiment was extracted with 8.5 per cent. sodium chlorid solution.

ANAPHYLACTIC STUDIES

The usual method used in demonstrating experimental anaphylaxis was employed. Two extracts of the pollen of *Ambrosia trifida* (giant ragweed) were prepared, *A* containing 8.5 per cent. sodium chlorid solution, and *B* 0.85 per cent. sodium chlorid solution, both with the dilution of 1 gm. of pollen to 10 c.c. of extracting solution. The nitrogen content of *A* was 1.61 mg. per cubic centimeter and of *B* 1.54 mg. per cubic centimeter.

Six guinea-pigs weighing from 150 to 500 gm. were injected intraperitoneally with 1 c.c. of solution *A* (1.61 mg. of nitrogen) diluted to 10 c.c. with water. After an interval of from eighteen to twenty-two days, from 5 to 10 c.c. of solution *B* (7.7 = 15.4 mg. of nitrogen) warmed to body heat was injected intraperitoneally. All the pigs showed varying degrees of such signs as restlessness, scratching of the nose

TABLE 1.—*Nonfatal Anaphylaxis in Guinea-Pigs with Pollen Extracts**

Animal	Wt., Gm.	Date of Injection	Amount Injected	Date of Shocking Injection	Amount Injected	Results
3	450	2/ 8/23	1 c.c. = 1.61 mg. N	2/27/23	5 c.c. = 7.7 mg. N	Slight symptoms
9	420	2/13/23	1 c.c. = 0.80 mg. N	3/ 5/23	10 c.c. = 15.4 mg. N	Slight symptoms
13	460	2/ 7/23	1 c.c. = 0.80 mg. N	2/26/23	10 c.c. = 15.4 mg. N	Slight symptoms
17	500	2/ 7/23	0.5 c.c. = 0.80 mg. N	2/27/23	5 c.c. = 7.7 mg. N	Slight symptoms

* Sensitizing and shocking doses given intraperitoneally. Similar results were obtained with other pigs.

TABLE 2.—*Nonfatal Anaphylaxis in Guinea-Pigs with Pollen Extracts**

Animal	Wt., Gm.	Date of Injection	Amount Injected	Date of Shocking Injection	Amount Injected	Results
5	430	2/21/23	1 c.c. = 1.61 mg. N	3/13/23	1 c.c. = 1.54 mg. N	Slight symptoms
11	450	2/21/23	1 c.c. = 1.61 mg. N	3/13/23	1 c.c. = 1.54 mg. N	Slight symptoms
19	300	3/ 7/23	1 c.c. = 1.61 mg. N	3/26/23	1 c.c. = 1.54 mg. N	Slight symptoms

* Sensitizing dose given intraperitoneally, shocking dose given intracardially. Similar results were obtained with other pigs.

and ears, elevation of the hair on the neck and back, urination, defecation, etc., but no death occurred. The normal pigs gave no evidence of a reaction (Table 1).

Six additional pigs were given a sensitizing dose similar to that used before, and after an interval of from eighteen to twenty-two days were injected intracardially with 1 c.c. of extract *B*. The results were similar to those obtained in the first group. No deaths occurred. The normal pigs gave no evidence of a reaction (Table 2).

After these failures to produce fatal experimental anaphylaxis with pollen extracts in guinea-pigs by the usual methods, we gave six young virgin guinea-pigs, weighing about 150 gm., two or three intraperitoneal

injections of from 2 to 3 c.c. of a fresh pollen extract (nitrogen, 1.32 mg. per cubic centimeter) at three day intervals and found that from eighteen to twenty-two days after the last injection typical contractions of the specifically sensitized uteri were obtained (Fig. 1). We then sensitized twelve young guinea-pigs, weighing about 150 gm., by the

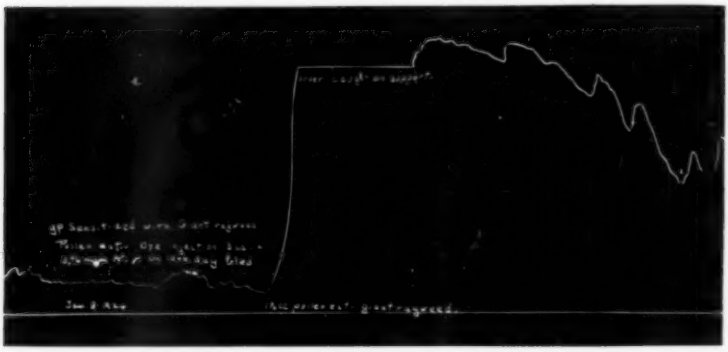

Fig. 1.—Record of active anaphylaxis to Ambrosia trifida (giant ragweed) pollen extract (uterine strip method) in guinea-pig.

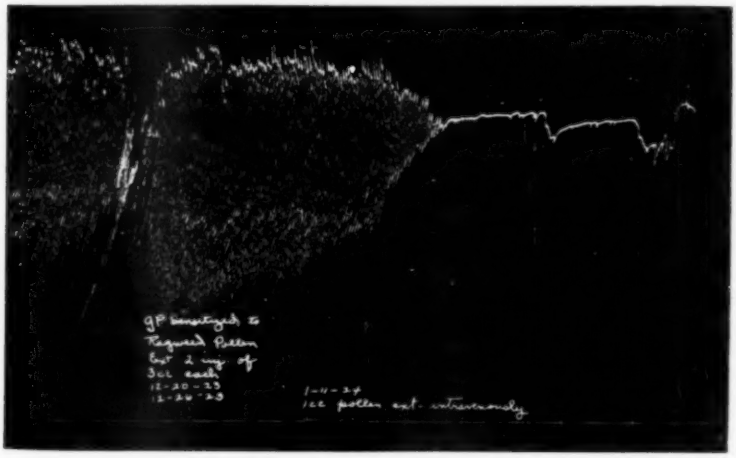

Fig. 2.—Record of positive bronchoconstriction in sensitized guinea-pig after injection of Ambrosia trifida (giant ragweed) pollen extract.

same method and after eighteen or twenty days injected from 1 to 2 c.c. of the pollen extract intravenously with the following results: Of six pigs that had received two sensitizing doses one died within eight minutes after receiving the injection, showing symptoms and postmortem

findings characteristic of anaphylaxis; four died before two hours with a gradually developing paralysis, and one, although very sick for a time, survived. Of six pigs that had received three sensitizing doses, three died within eight minutes after receiving the injection and three died within two hours. Of the twelve pigs, five died within eight minutes; six more died within two hours, and one pig survived. Four normal pigs of the same lot gave no symptoms following an intravenous injection of from 1 to 2 c.c. of the same pollen extract. We next sensitized six pigs weighing about 250 gm. by the same method and obtained in three pigs, after an intravenous injection of 1 c.c. of pollen extract, typical bronchoconstrictions as demonstrated by the graphic method reported in 1924 by Koessler and Lewis at the Buffalo meeting of the Association of Pathologists (Figs. 2 and 3). Normal pigs gave no reaction by this method.

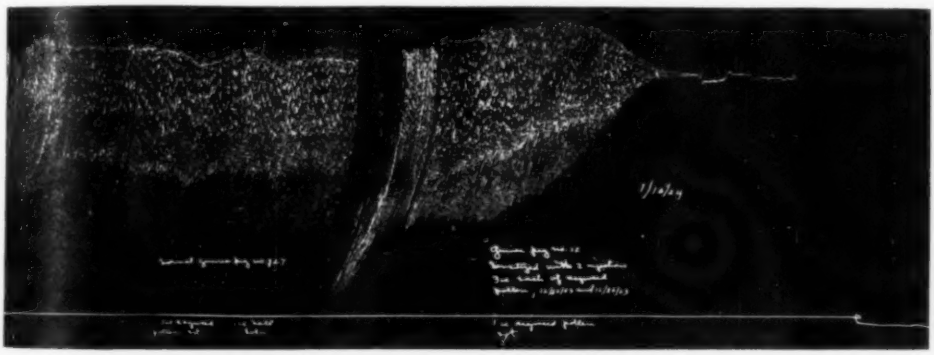

Fig. 3.—Record of bronchoconstriction and typical anaphylaxis after injection of Ambrosia trifida pollen.

As several reports of successful passive sensitization to pollens have appeared, we sensitized nine pigs by the method described above for this purpose. Nine days following the last injection, the pigs were bled and the blood serum was transferred to an equal number of normal pigs. These pigs were tested from twenty-four to forty-eight hours later by three methods: (1) Three pigs were injected intravenously with 1 c.c. of pollen extract and all were uncomfortable and restless for an hour or more and then appeared normal; (2) three virgin pigs were killed and their uteri gave typical, although moderate, contractions when tested with the pollen extract (Fig. 4), and (3) two of three pigs gave moderate bronchial contractions when injected intravenously with 1 c.c. of pollen extract.

ANTIBODY PRODUCTION

Four rabbits were given at three day intervals from twelve to twenty-five intravenous injections of *Ambrosia trifida* (giant ragweed) pollen extract prepared as described above. The first injection contained 0.5 mg. of nitrogen, and each succeeding injection was increased so that the twelfth contained 3.5 mg. of nitrogen. This dose was continued until the termination of the experiment. From five to six days after the last injection, the three surviving rabbits were bled and the serum tested for precipitins and complement fixating antibodies. The serum from all immunized rabbits contained precipitins for the pollen extract (Table 4) and showed complete inhibition of hemolysis by the complement fixation test. Normal rabbit serum gave negative results for both tests.

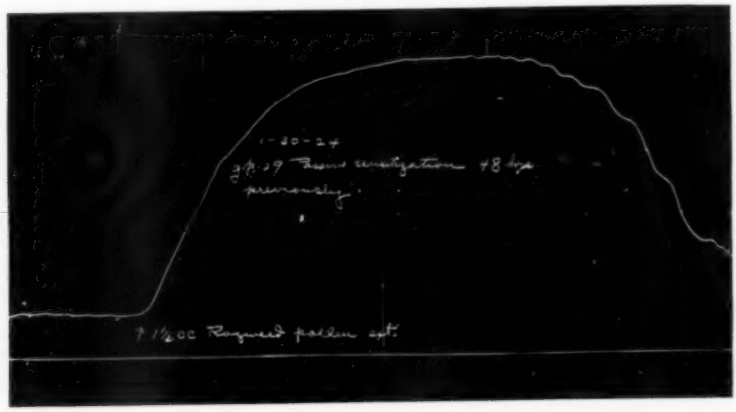

Fig. 4.—Record of passive anaphylaxis to Ambrosia trifida pollen extract, guinea-pig to guinea-pig (uterine strip method).

SPECIFICITY OF POLLENS

In 1873 Blackley [16] published the results of his extensive experiments to prove that pollens were the cause of the symptoms called "hay-fever" and that experimental contact showed a certain degree of specificity. He says:

> And I have also shown that, with very rare exceptions, this power is common, in some degree, to the pollen of almost all the plants experimented with. And although those belonging to the order *Graminaceae* have this property in a more marked degree than some, there are plants belonging to other orders which have it to almost, if not quite an equal extent.

16. Blackley, C. H.: Experimental Researches on the Cause and Nature of Hay-Fever, 1873.

Since Blackley's time the question of specificity of pollen has been studied extensively, not only by observing the effect of experimental contact, but also by other methods, such as ophthalmic reactions, skin sensitization and therapeutic tests. It is supposed that, if pollens from one member of a so-called group give a positive reaction in a sensitive person, all pollens from that group should also give positive reactions *regardless of whether the subject has ever had a previous contact with all of them.* Then, too, in discussions on the specific treatment of pollen disease it is commonly stated that extracts from any one pollen of a

TABLE 3.—*Fatal Anaphylaxis in Guinea-Pigs with Pollen Extracts* *

Animal	Wt., Gm.	Date of Injection	Amount Injected	Date of Shocking Injection	Amount Injected	Results
1	140	12/20/23 12/26/23	3 c.c. = 3.96 mg. N 3 c.c. = 3.96 mg. N	1/ 7/24	1 c.c. = 1.32 mg. N	Death in 26 minutes
3	150	12/20/23 12/26/23	3 c.c. = 3.96 mg. N 3 c.c. = 3.96 mg. N	1/10/24	1.5 c.c. = 1.98 mg. N	Death in 20 minutes
34	310	1/10/24 1/14/24 1/17/24	2 c.c. = 2.64 mg. N 3 c.c. = 3.96 mg. N 2 c.c. = 2.64 mg. N	2/ 5/24	1.5 c.c. = 1.98 mg. N	Death in 2 minutes
35	120	1/14/24 1/17/24	2 c.c. = 2.64 mg. N 2 c.c. = 2.64 mg. N	2/ 7/24	1.5 c.c. = 1.98 mg. N	Death in 2 minutes
40	180	1/14/24 1/17/24	3 c.c. = 2.96 mg. N 2 c.c. = 2.64 mg. N	2/ 8/24	2 c.c. = 2.64 mg. N	Death in 20 minutes
41	240	.....	.....	3/ 7/24	1.5 c.c. = 1.98 mg. N	No symptoms
42	200	.....	.....	3/ 7/24	1.5 c.c. = 1.98 mg. N	No symptoms

* Sensitizing doses given intraperitoneally, shocking doses intravenously. Similar results were obtained with other pigs.

TABLE 4.—*Production of Precipitins with Pollen Extracts in Rabbits* *

Rabbit	Date	Number of Injections: Subcutaneous	Intraperitoneal	Intravenous	Dilution of Pollen Extract: 30	60	120	240	480
2	5/6/24	0	0	0	—	—	—	—	—
2	8/7/24	0	0	21	++	++	+	—	—
3	5/6/24	0	0	0	—	—	—	—	—
3	8/7/24	0	0	16	+++	+++	++	+	—
4	5/6/24	0	0	0	—	—	—	—	—
4	8/7/24	2	2	12	++	++	+	—	—
5	8/7/24	0	0	0	—	—	—	—	—

* Tested before and after injections.

group are as efficacious as those from another member of the same group. The generally accepted explanation for this view is that the chief proteins of the pollens of a group are identical and that symptoms are produced by contact with that protein. In support of this view, Wells and Osborne [17] have definitely shown that, when tested anaphylactically, the chemical relationship of similar tissues of a group parallels the biologic relationship. However, a critical study of many of the

17. Wells, H. G., and Osborne, T. B.: J. Infect. Dis. **12**:341, 1913; J. Infect. Dis. **17**:259, 1915.

reports on pollen disease brings to light certain facts that cannot be disregarded. For instance, it has been observed that a certain percentage of all patients with pollen disease, tested in a routine manner with several pollens of a group, while reacting strongly to one or more of the pollens, react poorly or not at all to other pollens of the same group, and again that prophylactic treatment with one pollen extract does not yield the same therapeutic results as that with an extract of another member of the same group.

In recent reports, especially by Selfridge,[18] Watson and Kibler[19] and Bernton,[20] exception is taken to the unqualified acceptance of the view of group specificity. They agree that a certain amount of specificity exists, but contend that this conception does not explain satisfactorily many observed irregularities. Parker[21] has recently presented some experimental evidence to show that a definite specificity exists for the pollens of *Ambrosia elatior* and *Ambrosia trifida*.

With this question before us we began some studies over a year ago to learn more about the specificity of pollens. We found it difficult to evaluate much of the work already reported, as each investigator used his own methods in preparing materials and in making clinical and experimental comparisons. In our work we have used extracts of four of the common pollens, namely, *Ambrosia trifida* (giant ragweed), *Ambrosia elatior* (small ragweed), *Helianthus annuus* (sunflower) and *Zea mays* (corn) collected by one of us (H.L.H.) in the fall of 1922. An extract of each pollen was made by the method described above, and after the sterility was proved anaerobically and aerobically the nitrogen content was determined by the Kjeldahl method. With each pollen extract, six virgin guinea-pigs, about four weeks old and weighing not more than 150 gm., were given one intraperitoneal injection containing about 1.5 mg. of nitrogen. Twenty or more days later, the pigs were killed by a blow over the head; the uterus was removed at once and immersed until used in a modified Locke's solution which was kept at 37 C. and through which a constant stream of washed oxygen was bubbled. The uterine strips were then tested by the Dale method with the nonspecific and specific extracts. Dilutions up to 1 : 5,000 with a nitrogen content of about 0.002 mg. per cubic centimeter, except as noted below, gave typical contractions as soon as the specific extract was added, while with the exception of the extract of *Zea mays* pollen much lower dilutions gave no reaction with the normal uterine strip.

18. Selfridge, G.: Calif. State J. Med. **17**:106 (April) 1919; ibid. **17**:139 (May) 1919.

19. Watson, S. H., and Kibler, C. S.: Etiology of Hay-Fever in Arizona and Southwest, J. A. M. A. **78**:719 (March 11) 1922.

20. Bernton, H.: Treatment of Seasonal Hay-Fever, and Possible Causes of Failure, J. A. M. A. **80**:1301 (May 5) 1923.

21. Parker (Footnote 10, second reference).

When tested with the nonspecific extract the sensitized uteri gave little or no response, except to the extract of *Zea mays* pollen. The pollen from the two ambrosias gave small nonspecific reactions when tested against each other and marked reactions when tested specifically (Figs. 5 and 6). This difference has been noted many times clinically while making the routine skin sensitization tests with these two pollens. All uterine strips, normal, nonspecifically sensitized and specifically sensi-

Fig. 6.—Record of active anaphylaxis to Helianthus annuus (sunflower) pollen extract in guinea-pig (uterine strip method).

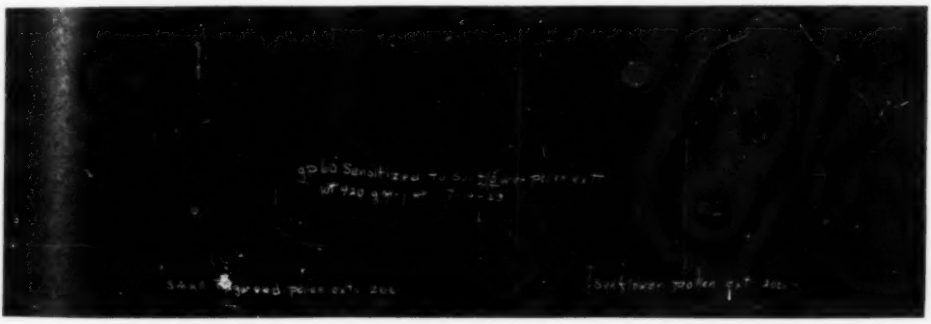

Fig. 6.—Record of active anaphylaxis to Helianthus annuus (sunflower) pollen extract in guinea-pig (uterine strip method).

tized reacted vigorously to the extract of *Zea mays* pollen in dilutions as high as 1 : 30,000 or about 0.0002 mg. of nitrogen per cubic centimeter (Fig. 7). Clinically we have noted also that many patients at least give small positive skin reactions to the pollen of *Zea mays* regardless of whether they give reactions to any other pollen. Corn pollen contains therefore an oxytocic principle.

COMMENT

This experimental evidence, showing that by using pollen extracts as an antigen characteristic anaphylactic reactions can be elicited in the guinea-pig and that antibodies can be demonstrated in the blood of properly sensitized experimental animals, strengthens the view that pollen disease may be placed in the group of anaphylactic disorders, and suggests that this phase of the problem should be considered by the clinician when he institutes prophylactic measures for the relief of symptoms. Our experience, as well as that reported by others, shows that the sensitization of animals with pollens is more difficult to accomplish than with proteins from most other sources, and this may explain the unsuccessful attempts of some investigators to demonstrate either antibodies or anaphylactic phenomena. These failures may be due not only to the technic used in sensitizing animals but also to the method

Fig. 7.—Record of uterine reaction to Zea mays (corn) pollen extract in normal guinea-pig.

of preparing the extracts which are to be used as antigen. Most of the successful workers, except Harrison and Armstrong, who use a glycerol and sodium chlorid solution mixture, have used different concentrations of plain or slightly modified solutions of sodium chlorid. It is also of great importance to select pollens of known origin and purity as the microscopic examination of specimens obtained from various sources often reveals the presence of different pollens or other contaminations. The indicated specificity of pollens is of great importance clinically, as was again pointed out recently by Bernton, and when generally recognized should make the prophylactic treatment of pollen disease more satisfactory. We recommend that efforts be made to educate the public

to the importance of making accurate observations of the appearance and disappearance of symptoms. These observations, a knowledge of the pollinating season for all local "hay-fever" plants, and information obtained from carefully controlled skin tests make possible a more accurate diagnosis on which to a large extent depends the degree of success of treatment.

At present the evidence for placing pollen disease in the category of anaphylactic phenomena is the following:

1. Symptoms produced in sensitive individuals by the inhalation of minute quantities of the specific pollen.

2. Anaphylactic-like symptoms occasionally produced in sensitive persons by the injection of minute quantities of specific pollen extracts.

3. Desensitization of sensitive persons by repeated injections of small doses of pollen extracts.

4. Demonstration of specific antibodies in the blood of man during seasonal attacks.

5. Frequent association with other disorders of suspected or known anaphylactic nature.

6. Presence of positive skin and ophthalmic reactions with specific pollens or pollen extracts.

7. Experimental demonstration of the antigenic property of pollens.

8. Moderate blood eosinophilia.

9. Hypotension.

CONCLUSIONS

1. If suitable procedures are followed the antigenic property of pollens can be demonstrated in specifically sensitized animals by: (*a*) production of characteristic fatal anaphylatic phenomena; (*b*) positive uterine strip reaction; (*c*) positive bronchoconstrictions, and (*d*) production of specific antibodies giving precipitin, complement fixation and passive sensitization reactions.

2. The response of sensitized uterine strips to *Ambrosia trifida* and *Ambrosia elatior* and to *Helianthus annuus* is specific.

3. The demonstration of the antigenic property of pollens while not itself proving the anaphylactic nature of pollen disease is highly significant for this interpretation.

4. *Zea mays* pollen contains an oxytocic principle.

THE EFFECT ON PARAMECIA OF BLOOD SERUMS, ESPECIALLY FROM PATIENTS WITH CARCINOMA *

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Many studies on the blood of cancer patients have been made in an effort to determine the presence of a specific substance which is characteristic of the disease and which can be detected soon enough to aid early diagnosis. The tests that have been proposed have involved the assumption that the blood of cancer patients was chemically different from that of normal subjects or of those with other diseases, or that a specific cytolysin, enzyme, hemolysin or antibody was present. These tests have proved unsatisfactory either in not being specific for cancer, as has been shown in the study of the phosphorus content of the blood,[1] or as involving so many variable factors as to invalidate them. Underhill and Woodruff [2] found that an extract of cancer tissue contained a specific substance that was lethal to paramecia. This observation—that there was a substance in cancer tissue different from anything in normal tissue in its effect on living cells—was confirmed by Calkins [3] in a study of cancer tissue extract on didinium.

The problem of a specific substance in the blood of cancer patients is still a matter to be investigated. The present work deals with one phase of this problem, experiments having been conducted to determine the presence or absence of a substance in serums from cancer and from other patients which may affect paramecia differently from normal serums. This biologic aspect of the blood in cancer has not received as much attention as the morphologic, physical and chemical phases of the question. Various methods have been devised for studying growth promoting and growth inhibiting substances. Carrell [4] has shown that leukocyte extracts may accelerate a growth of tissues in vitro. Macht

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* From the medical service of the Collis P. Huntington Memorial Hospital of Harvard University.

* This paper is No. 44 of a series of studies in metabolism from the Harvard Medical School and allied hospitals. The expenses of this investigation have been defrayed, in part, by a grant from the Proctor Fund of the Harvard Medical School for the Study of Chronic Diseases.

1. Buckman, T. E.; Minot, G. R.; Daland, G. A., and Weld, M.: Blood Phosphorus; Its Relation to Cancer and Anemia, Arch. Int. Med. **34**:181 (Aug.) 1924.

2. Underhill, F. P., and Woodruff, L. L.: J. Biol. Chem. **15**:401, 1913.

3. Calkins, G. N.: J. Cancer Res. **1**:399 (Oct.) 1916.

4. Carrell, Alexis: J. Exper. Med. **36**:385 (Oct.) 1922.

and Lubin [5] demonstrated that variations of growth of lupin seedlings occurred after serums from patients with various conditions were added to the nutrient mediums. Similar unpublished observations have been made in this laboratory.

Paramecia have been used widely as an index of the growth promoting and destructive action of substances, such as the toxicity of alcohol, the effect of thyroxin and of ultraviolet light. Several investigators have reported work on the influence of serums on paramecia. Takenouchi [6] found that serums from cold blooded animals were more lethal for paramecia than those taken from warm blooded animals. Ledoux-Lebard [7] has shown that normal human serums are less toxic to these infusoria than those from animals. He also studied a few serums from sick people and observed that some were more, others less toxic than those from normal subjects.

MATERIAL AND METHODS

Venous blood was withdrawn from the person to be studied, allowed to clot at room temperature and then centrifugalized. The serum was removed to a test tube, kept stoppered to prevent evaporation, and its effect on paramecia was tested on the same day that it was obtained.

The paramecia were kept on hollow ground glass slides in a moist chamber. Daily isolations were made in order to maintain a regular division rate and to observe the condition of the organisms, in accordance with standard methods. Slide cultures from which the stock organisms had been removed three or four days previously were used in the experiments. Thus, the cultures had enough organisms in them with which to work and were approximately of the same concentration of paramecia and of similar bacterial content.

The dilutions of paramecia with serum were made in a blood corpuscle counting pipet so that the proportion of serum and fluid containing paramecia could be controlled. In most of the experiments a dilution of 25 per cent. culture and 75 per cent. serum was used. This was varied for a study of dilution effects. The culture was drawn up into the pipet and then the serum; the whole amount was blown out on a glass slide, then redrawn into the pipet and expelled a second time. In this way the paramecia were thoroughly mixed with the serum. This drop of material was surrounded by a ring of petrolatum, covered with a glass cover slip and examined under the microscope.

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5. Macht, D. I., and Lubin, D. S.: Proc. Soc. Exper. Biol. & Med. **20**:265, 1922-1923; J. Pharm. & Exper. Therap. **22**:413 (Jan.) 1924.

6. Takenouchi, M.: J. Infect. Dis. **23**:396 (Nov.) 1918.

7. Ledoux-Lebard, M.: Ann. de l'Inst. Pasteur **16**:510, 1902.

There are many ways of determining the death rate of infusoria, but in this study vesicle formation has been chosen as an index of cytolysis.[8] This can be observed easily under low power of the microscope and on a number of organisms in the chamber at one time. Vesicle formation can be detected with greater accuracy than the cessation of the cilia beat, or the time at which the paramecia will no longer respond to electrical stimuli. The vesicles are blister-like elevations which, according to Chambers,[9] appear on the surface of the paramecia when they are placed in an abnormal environment. The vesicles appear to be beneath the pellicle but are external to the protoplasmic surface. Observations were made on from fifteen to forty organisms with each serum tested. The time at which the organisms were mixed with the serum and also the time when the first vesicle appeared on each organism in the chamber were recorded. From this data the percentage of the total number of organisms forming vesicles in a given period of time could be found. The frequency curves of the rate of vesicle formation have been studied in one, three and five minute intervals, as has also the summation of the frequencies.

The five minute interval was chosen for final analysis as it seemed to minimize the differences obtained with normal serums of the same subject from day to day, any errors of observation and manipulation, and the differences due to the daily variation in the resistance of the organisms, except during periods of endomixis.

A pathologic and a normal serum usually were studied on the same day. The comparison of the effects on the same day is especially valuable, even more so than the comparison of results obtained at times quite distant from each other, as differences may be the result of changes in the resistance of the paramecia.

Some twenty-seven observations were made on the serums of nine different normal subjects and also six observations on two of these at the time of menstruation.

Eighteen cases of carcinoma were studied. These included tumors of the breast, palate, rectum, throat, mouth, esophagus and eye. The cases represented many phases of the disease; some were early cases and others were in a terminal, toxic stage. As control observations the serums of patients with diseases other than cancer were studied. These cases included four cases of chronic myelogenous leukemia, two of chronic hemolytic jaundice, five of eclampsia, two of uremia due to chronic nephritis and one case each of the following: chronic lymphatic leukemia, lymphosarcoma, melanotic sarcoma, obstructive jaundice (cause undetermined), toxic jaundice from arsphenamin poisoning, pelvic sepsis, hydroa aestivale, and pernicious anemia in a stage of relapse.

8. Bovie, W. T., and Daland, G. A.: Am. J. Physiol. **66**:55 (Sept.) 1923.
9. Chambers, R.: General Cytology, edited by E. Cowdry, Univ. Chicago Press, 1924, p. 253.

EFFECT OF NORMAL SERUMS ON THE RATE OF VESICLE FORMATION IN PARAMECIA

The percentage of the total number of organisms forming vesicles in a given frequency interval varies in normal subjects. The height of the frequency curve with each normal serum studied occurred in the frequency interval of from six to ten minutes. For purposes of comparison this interval is used. The mean value for the percentage of the total number of organisms forming vesicles in this period is 63.7 per cent. This figure may vary from 35 to 80 per cent., or approximately ± 25 per cent. from the mean. These figures represent the results on different days over a period of several months. From data at hand it seems evident that on a given day the variation between the effects of normal serums on paramecia is not so great.

TABLE 1.—*Effect of Blood Serums from Normal Subjects and from Patients with Carcinoma on the Rate of Vesicle Formation in Paramecia*

Frequency Interval in Minutes	Percentage of the Total Number of Paramecia Forming Vesicles in the Given Interval	
	Mean Values for Fourteen Normal Serums, per Cent.	Mean Values for Eleven Carcinoma Serums, per Cent.
1 to 5	7.6	9.6
6 to 10	63.7	61.9
11 to 15	16.0	18.7
16 to 20	3.2	5.2
21 to 25	3.7	1.1
26 to 30	1.5	0.8
31 to 35	2.0	0.9
36 to 40	1.4	0.0
41 to 45	0.3	0.0
46 to 50	0.3	0.8
51 to 55	0.0	0.8
56 to 60	0.0	...
61 to 65	0.3	...

The mean values for the percentage of the total number of paramecia forming vesicles in each frequency interval for fourteen comparable tests on six different subjects are given in Table 1.

EFFECT ON PARACEMIA OF BLOOD SERUMS FROM CASES OF CARCINOMA

The mean values for observations that are comparable for eleven cases of carcinoma are given in Table 1. They are very nearly the same as those in this table for the normal persons. Like normal serums, the lethality of the serums from patients with carcinoma varied. The magnitude of this variation is ± 22 per cent., or approximately of the same degree as that of the normal series. Comparable tests of cancer and normal serums on a given day, not comparable with those made on different days, further indicated no difference in the effect of carcinoma and normal blood serums on paramecia. The carcinoma serum was sometimes slightly more toxic than the normal and sometimes less so. The difference was never striking.

EFFECT ON PARAMECIA OF THE SERUMS FROM OTHER PATHOLOGIC STATES AND DURING MENSTRUATION

Serums from patients other than those with cancer may affect paramecia differently from normal serums. It is to be recalled that comparable tests on different days showed normal serums to cause, at the greatest a ± 25 per cent. variation in the rate of vesicle formation and that tests on the same day showed less variation. The serums from some patients caused the percentage of the total number of organisms forming vesicles in the six to ten minute interval to be 50 per cent. greater than the normal serums tested on the same day (one case of chronic lymphatic leukemia and one of chronic myelogenous leukemia in remission), and sometimes 50 per cent. less (one of melanotic sarcoma, one of eclampsia, and one of obstructive jaundice). Other serums, those from one case each of toxic jaundice, sepsis, pernicious anemia and eclampsia, were from 15 to 25 per cent. more toxic than normal serums obtained the same day.

The results obtained in normal women during menstruation are not included as "normal" because it was demonstrated that blood serum during this period was about 20 per cent. more toxic for paramecia than at other times. These results are in accord with those of Macht and Lubin [5] on the effect of serum "menotoxin" on the growth of lupin seedlings and also with that of Herz and Weichbrodt,[10] who have confirmed their work.

EFFECT ON PARAMECIA OF ALTERATIONS OF THE PHYSICAL AND CHEMICAL PROPERTIES OF SERUMS

The differences in the effect on paramecia of blood serums cannot be attributed to a disease per se, but rather to some properties which may or may not be manifestations of disease, much as is the case for the rate of sedimentation of red blood corpuscles. The observations of Takenouchi [6] and Ledoux-Lebard [7] also suggest that the differences in the reaction of paramecia to serums from cold and warm blooded animals and from man are due to variations in the physical and chemical composition of the serums.

Further experiments, in which serums have been altered by dilution, presence of hemoglobin, heat and irradiation, indicate that alterations of physical and chemical properties do influence the lethal effect of serums on paramecia.

Effect of Changing Concentration of Serum.—It is to be expected that the physical properties of the surrounding medium will be factors in the rate of vesicle formation. Any change in the concentration of

10. Herz, E., and Weichbrodt, R.: Deutsch. med. Wchnschr. **50**:1210 (Sept. 5) 1924.

the medium will produce a reaction in the organism, the intensity of the reaction varying with conditions. This may be illustrated by observations on the mode and rate of cytolysis with different dilutions of the same serum.

In a concentrated combination of serum and infusion the organism is first stimulated, swimming very rapidly. This is followed by the avoiding reaction, a slowing up process, cessation of motion, vesicle formation and sometimes rupturing, with or without previous vesicle formation.

In a weaker concentration of serum the sequence of the reactions is practically the same. Sometimes, however, instead of forming vesicles, the usual index of cytolysis, the organisms become opaque; this seems to be due to internal disorganization of the protoplasm.

This series of reactions usually takes place in two minutes with 90 per cent. serum solution or in about eighty minutes with 25 per cent. serum solution. The results with different dilutions of serum are given in Table 2.

TABLE 2.—*Effect on Paramecia of Changing the Concentration of the Serum* *

Concentration of Serum, per Cent.	Frequency Interval in Five Minute Periods (1-5, 6-10 Minutes)													
	5	10	15	20	25	30	35	40	45	50	55	65	70	80
25	0.0	0.0	7.4	3.7	7.4	0.0	14.8	14.8	14.8	7.4	11.1	7.4	7.4	3.7
50	11.5	19.2	11.5	19.2	11.5	7.6	3.8	3.8	0.0	0.0	3.8	0.0	7.6	0.0
75	15.8	63.0	10.4	5.2	5.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

* The figures in the columns represent the percentage of the total number of organisms forming vesicles in the given frequency interval.

The effect of dilution of normal serums was comparable with that of similarly diluted cancer serums. In some other pathologic conditions the 75 per cent. serum solution was more lethal than the same concentratoin of normal serum and equivalent to a greater concentration (90 per cent.) of normal serum. Thus, by varying the concentration of serums the difference between normal and abnormal serums can be clearly shown.

Effect of Presence of Hemoglobin in Serum.—It was observed early in this study that serums in which hemoglobin was evident were less toxic to paramecia than serums without hemoglobin. Hence tests recorded above were made with serums free from hemoglobin. Several experiments were done with serums containing hemoglobin liberated mechanically from the red cells. Such a serum was distinctly less toxic than a sample free of hemoglobin from the same subject. A typical experiment is recorded in Table 3.

Effect of Heated Serums.—Serum heated in a water bath at 60 degrees C. for ten minutes and then cooled also was found to be

decidedly less toxic than an unheated sample of the same serum. Data in Table 3 illustrate this effect.

Effect of Irradiated Serums.—Alteration in the physical and chemical properties of serums also may be produced by irradiation. Roffo and Correa [11] have shown that the surface tension and viscosity of the blood may be thus increased.

In experiments in this laboratory serum was irradiated in a quartz test tube 70 cm. from a Cooper-Hewitt air cooled mercury vapor lamp for a period of one hour. The irradiated serum was definitely less toxic than the nonirradiated control. A sample experiment is shown in Table 4. The results are in accord with those of similar experiments by Macht and Hill [12] on the effect of irradiated plasma on the growth of lupin seedlings.

TABLE 3.—*Effect on Paramecia of the Same Serum Before and After Heating and Also After the Liberation of Hemoglobin from the Red Cells into the Serum* *

Serum, 75 per Cent. Solution	Frequency Interval in Five Minute Periods (1-5 Minutes, etc.)													
	5	10	15	20	25	30	35	40	50	55	65	70	75	85
Unchanged	9.1	54.5	18.1	9.1	0.0	0.0	0.0	0.0	0.0	9.1	0.0	0.0	0.0	0.0
Tinged with hemoglobin	0.0	10.3	68.8	10.3	3.4	0.0	3.4	3.4	0.0	0.0	0.0	0.0	0.0	0.0
Previously heated to 60 C. for 10 minutes	0.0	8.3	16.6	0.0	16.6	8.3	8.3	0.0	8.3	0.0	8.3	8.3	8.3	8.3

* The figures in the columns represent the percentage of the total number of organisms forming vesicles in the given frequency interval.

TABLE 4.—*Effect on Paramecia of Blood Serum Irradiated with Ultraviolet Light* *

Serum	Frequency Interval in Five Minute Periods (1-5 Minutes, 6-10 etc.)									
	5	10	15	20	25	30	35	40	45	55
Nonirradiated	3.7	33.3	14.5	11.1	11.1	7.4	14.8	0.0	3.7	0.0
Irradiated 1 hour	0.0	0.0	17.6	11.8	17.6	17.6	29.4	0.0	0.0	5.9

* The serum was irradiated in a quartz test tube, 70 cm. from a Cooper-Hewitt air cooled, mercury vapor lamp for one hour. The figures in the columns give the percentage of the total number of organisms forming vesicles in a given frequency interval.

The serums from three patients with carcinoma were studied before and immediately after roentgen-ray treatment with the high voltage, constant potential, short wave length (0.185 angstrom units) apparatus described by Duane [13] (the dose in these cases was about 600 electrostatic unit seconds, target distance 80 cm. and filter one-half millimeter copper). The difference in the lethal effect of the serums was not marked, but in each case the serum after irradiation was less toxic to the paramecia. The results of two experiments are given in Table 5.

11. Roffo, A. H., and Correa, J.: Semana méd. **27**:17 (July 3) 1924.
12. Macht, D. I., and Hill, E.: J. General Physiol. **6**:671 (July) 1924.
13. Duane, William: Am. J. Roentgenol. **9**:781 (Dec.) 1922.

Thus, while therapeutic roentgen-ray irradiation causes various changes in the blood, including a decreased toxicity of serum for paramecia, this change is not specific, but is comparable apparently to alterations produced by disease, dilution, presence of hemoglobin, heat, and irradiation with ultraviolet light.

CONCLUSIONS

1. Paramecia when mixed with human blood serum form vesicles, the rate of formation being an index of the lethality of the serum.

TABLE 5.—*Effect on Paramecia of Serums from Patients Before and Directly After Roentgen-Ray Treatment* *

	Roentgen Ray	Frequency Interval in Five Minute Periods (1-5, 6-10, etc.)					
		5	10	15	20	25	30
Carcinoma of breast	Before	0.0	48.3	29.7	16.1	6.0	0.0
	After	0.0	27.0	31.5	31.5	4.5	4.5
Carcinoma of larynx	Before	0.0	54.6	18.2	18.2	0.0	9.0
	After	0.0	38.4	15.4	7.7	30.4	7.7

* The patients received roentgen-ray treatment with a high voltage, constant potential, short wave length apparatus (dose, 600 electrostatic unit seconds; target distance, 80 cm., and filter, one-half millimeter copper). The figures in the columns represent the percentage of the total number of organisms forming vesicles in the given frequency interval.

2. No specific substance was found in the blood serum of patients with carcinoma which affected the rate of vesicle formation in paramecia in any way different from normal serum.

3. Serums from diseased persons may resemble those from normal subjects in their effects on paramecia, or their toxicity may be greater or less. Serum obtained at the time of menstruation is slightly more toxic than at other times.

4. Serums differing in their effects on paramecia may be made to act similarly by altering their concentration.

5. Diluted serums, serums containing hemoglobin or that have been heated, serums irradiated with ultraviolet light, and serums of patients who have just received roentgen-ray therapy are all less toxic for paramecia than the control serums.

DIABETES

ELECTROCARDIOGRAPHIC STUDIES *

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For many years it has been known that diabetic patients frequently have marked vascular disturbances, particularly of the lower extremities. Heitz [1] has recently shown that all of fifty-three diabetic patients showed some definite vasomotor changes, such as cold feet, edema or cyanosis, while thirty-five, or 66 per cent. of the same group, had beginning arteritis, as shown by oscillometry. Joslin [2] states that 15.1 per cent. of diabetic patients in his series of 3,000 cases died of cardiovascular changes, while 2 per cent. of this number developed gangrene. He quotes Buerger's [3] statement that diabetic gangrene shows typical lesions of atherosclerosis or arteriosclerosis. "These lesions differ in no way from the lesions of the arteries in arteriosclerotic or senile gangrene, and justify the conclusion that diabetic gangrene is due to an atherosclerotic or arteriosclerotic process." This statement has been generally accepted, but no statement has been found in the literature as to the exact etiology of this arteriosclerosis. Fitz and Murphy [4] state that "cardiovascular or renal disease, including gangrene, often causes death in diabetics. Unfortunately cardiovascular and renal disease are very common in people past the middle period of life." These authors, however, give no more light on the etiology of these changes.

No definite statements about the pure cardiac changes have been found in the literature, nor have a series of electrocardiograms of diabetic patients been published. The only electrocardiographic work on animals seems to be that of Belehradek and Noyons,[5] who perfused the excised hearts of frogs with pure anhydrous glucose solutions. Electrocardiographic tracings of these isolated hearts were taken at

* From the Cardiographic Laboratory, the Investigative Medical Service, and the Otto Baer Fund for Clinical Research of the Michael Reese Hospital.

1. Heitz, J.: Circulatory Derangement in the Legs in Diabetes, Médecine **4**:783 (July) 1923.

2. Joslin, E. P.: Treatment of Diabetes Mellitus, Ed. 3, Philadelphia, Lea and Febiger, 1923.

3. Buerger, L.: The Pathology and Diagnosis of So-Called Diabetic Gangrene, Arch. Diag. **8**:101 (April) 1915.

4. Fitz, R., and Murphy, W. P.: Cause of Death in Diabetes Mellitus, Am. J. M. Sc. **168**:313 (Sept.) 1924.

5. Belehradek, J., and Noyons, A. K. M.: L'electrocardiogramme du cœur perfuse au glucose, Compt. rend. Soc. de biol. **88**:621 (March 10) 1923.

different times during the perfusion. A preliminary period of heart block (complete arrest) was observed; then there was a change in the action current, as shown by an increased amplitude of the main deflection of the galvanometer string.

Our studies were undertaken to see in what way electrocardiographic tracings of diabetic patients differ from those of normal persons of the same age and sex. Furthermore it seemed desirable to ascertain the influence of changes in the blood sugar content on the electrocardiographic tracings.

METHOD

A series of electrocardiograms were taken on diabetic patients with known blood sugar concentrations. A Cambridge type of electrocardiographic instrument, a modification of the Einthoven string galvanometer, was used. In each case all three leads of the electrocardiogram were taken with the patient lying down. Each tracing was accurately standardized, 1 cm. swing of the string on the camera being equivalent to 1 millivolt of current. This was the usual hospital routine. Clinical interpretations were made, and the findings carefully noted.

FINDINGS

In the course of several months it was found that a considerable number of electrocardiograms of diabetic patients showed changes that were more marked than the physical findings of the patients seemed to warrant. Tracings were taken of seventy diabetic patients in the hospital, with known blood sugar concentrations. Of these seventy, twenty-one, or 30 per cent., showed notching of the Q-R-S complex in one or more leads. In no case included in this group did Q-R-S measure 0.1 second or more. For this reason it would be difficult to say that there was a true bundle branch block or other anatomic lesion of the conducting pathways in these cases. In all cases, the P-R interval was within normal limits, 0.2 second or less. In all cases the ventricular rate was over 58 per minute. No cases were included in which the diabetes was accompanied by grave cardiac changes, as auricular fibrillation, flutter, idioventricular rhythm, angina pectoris, marked decompensation or aneurysm.

In this series of seventy diabetic patients, forty had high blood sugar concentrations at the time the tracings were taken. The concentration varied between 210 mg. and 460 mg. of sugar per hundred cubic centimeters of blood, the average being 282 mg. Twenty patients had blood sugar concentrations between 130 and 190 mg., with an average of 150 mg., while ten showed concentrations below 130 mg.

Notching was more frequent in the curves of patients with high blood sugar concentrations than in those with lower concentrations. No

notching occurred in any of the ten electrocardiograms taken of patients with blood sugar concentrations of less than 120 mg. per hundred cubic centimeters of blood. When the sugar concentration varied between 130 and 190 mg. only five of the twenty cases, or 25 per cent., showed notching of Q-R-S in any of the three leads. With higher sugar concentrations, 210 mg. or more, notching occurred much more frequently in thirteen of forty cases, or in 32 per cent.

The swing of the galvanometer string was higher in cases with high blood sugar concentrations than in those with lower concentrations. When the sugar concentration was 210 mg. or higher, the average standardized deflection in all the forty cases was 10.7 mm. in Lead I, 9.5 mm. in Lead II, and 8.5 mm. in Lead III. When these figures were more carefully examined it was found that this group could be subdivided into two groups. In the first group were included all those curves which showed no notching in any of the three leads, and here the average swing in the first lead was 11.6 mm.; in the second lead, 10.5 mm., and in the third lead, 9.8 mm. In the second group were included all of the curves which had a notched Q-R-S in any of the leads, and here in Lead I the average main deflection was 8.4 mm.; in Lead II, 7.0 mm., and in Lead III, only 4.9 mm. There was a marked increase in the notching in Lead III as compared with Lead I.

In the group of twenty diabetic patients with lower blood sugar concentrations, i. e., between 130 and 190 mg. of sugar, the average being 150 mg., the average swing for all twenty curves was 11 mm. in Lead I, 8.8 mm. in Lead II, and 8.3 mm. in Lead III. When only those curves were included in which there was no notching in any of the three leads, the average main deflection in Lead I measured 11 mm., in Lead II, 7.2 mm., and in Lead III, 7.5 mm. When only those cases were included in which notched curves were included, the average main deflection in Lead I was 10.1 mm., in Lead II, 8.6 mm., and in Lead III, 5.2 mm.

In the group of ten diabetic patients with blood sugar concentrations below 120 mg., none of the curves showed any evidences of notching. The average main deflection in Lead I was 8.3 mm., in Lead II, 8.3 mm., and Lead III, 8.8 mm.

CONTROL TRACINGS

Control tracings were made of two different groups. The first group consisted of thirty normal men and women approximately the same age as the diabetic patients. These were selected from the hospital personnel and were all persons in good physical condition. The second group was selected from among the patients in the hospital who had approximately the same physical findings as the diabetic patients, excluding the diabetes, i. e., nephritis, myocarditis, hypertension, arteriosclerosis and the like. In the first group, the normal controls, four

curves were notched, but only in the third lead, none showing notching in Leads I or II. The general average for all curves was 7.6 mm. in Lead I; in Lead II, 8.4 mm., in Lead III, 5.9 mm. For all curves, excluding notched ones, the average swing in Lead I was 7.3 mm.; in Lead II, 8.4 mm., and in Lead III, 6.4 mm. For the four curves which showed notching in Lead III, the average for the first lead was 8.7 mm.; for the second lead, 8.5 mm., and for the third lead, 4.7 mm.

In the second group of controls three of the twenty, or 15 per cent., showed definite notching in one or more leads. The average main deflection for all curves was: in Lead I, 7 mm.; in Lead II, 8.2 mm., and in Lead III, 5.7 mm. When curves that showed notching in any of the three leads were excluded, the main deflection in Lead I measured 8.1 mm.; in Lead II, 8.8 mm., and in Lead III, 6.5 mm. When only those curves were included which showed definite notching, the main deflection in Lead I measured 7 mm.; in Lead II, 5.6 mm., and

TABLE 1.—*Average Deflections in Diabetic and Control Cases*

Sugar Concentration	R. Lead I	R. Lead II	R. Lead III	Remarks
210 mg. or more	10.7 mm.	9.5 mm.	8.5 mm.	All cases (40 cases)
	11.6 mm.	10.5 mm.	9.8 mm.	No notching (27 cases)
	8.4 mm.	7.0 mm.	4.9 mm.	All notched (13 cases)
130 to 190 mg.	11.0 mm.	8.8 mm.	8.3 mm.	All cases (20 cases)
	11.0 mm.	7.2 mm.	7.5 mm.	No notching (15 cases)
	10.1 mm.	8.6 mm.	5.2 mm.	All notched (5 cases)
Under 130 mg.	8.3 mm.	8.3 mm.	8.8 mm.	All cases, none notched (10 cases)
Normal controls	7.6 mm.	8.4 mm.	5.9 mm.	All cases (30 cases)
	7.3 mm.	8.4 mm.	6.4 mm.	No notching (26 cases)
	8.7 mm.	8.5 mm.	4.7 mm.	All notched in Lead III (4 cases)
Control	7.0 mm.	8.2 mm.	5.7 mm.	All cases (20 cases)
	8.1 mm.	8.8 mm.	6.5 mm.	No notching (17 cases)
	7.0 mm.	5.6 mm.	3.0 mm.	All notched (3 cases)
All controls	7.3 mm.	8.3 mm.	5.8 mm.	All cases (50 control cases)

in Lead III, only 3 mm. The general averages for all control curves were: in Lead I, 7.5 mm.; in Lead II, 8.3 mm., and in Lead III, 5.8 mm. When all curves that showed notching in any of the three leads were excluded, the main deflection in Lead I measured 7.6 mm.; in Lead II, 8.3 mm., and in Lead III, 6.4 mm. In the case of the curves that showed notching in one or more leads, Lead I measured 8 mm.; Lead II, 7.2 mm., and Lead III, 3.9 mm.

From Table 1 it is seen that the swing of the galvanometer string was consistently and constantly greater in all groups of diabetic patients than in any or all control groups.

Since diabetic patients with high sugar concentrations showed a greater deflection of the galvanometer string than those with lower concentrations, it was thought advisable to see if changes in the blood sugar concentration would produce changes in the electrocardiographic tracings. For this reason electrocardiographic tracings were taken of a

special group of six diabetic patients throughout the course of treatment in the hospital, when the blood sugar was at different levels. Since there was always considerable variation in the amount of sugar present in the circulating blood at different times of the day, electrocardiographic tracings and blood sugar estimations were made at approximately the same hour each day. The tracings were taken about two hours after breakfast, and two and one half hours after insulin when this was used. As soon as the electrocardiogram was completed, blood was drawn for the sugar estimation. In this way the amount of sugar present at the time the tracing was made was known.

All of the six patients were old diabetic patients, i. e., the disease had been recognized in each case for at least three years. No patient was in a serious condition; none showed evidence of marked acidosis, coma or other complications outside the diabetes. In all cases there was a high initial fasting blood sugar, at least 230 mg. of sugar per hundred cubic centimeters of blood. All the patients were kept at a high sugar level for several days, and electrocardiograms and sugar estimations were made daily. This was done in order to get a standard tracing to judge from. At the end of four days, dietetic treatment was begun. The blood sugar dropped gradually. During this period electrocardiograms and blood sugar estimations continued to be made daily. In two cases the blood sugar remained at a high level in spite of dietetic management so that in these two patients insulin was used.

In each case, as the concentration of the blood sugar decreased there was a decrease in the height of the main deflection, except in those cases in which R was notched to begin with. In these cases, R tended to increase in height as the blood sugar decreased, in other words there was a diminution in the notching. The change in height of Q-R-S was fairly rapid, and paralleled the sugar concentration quite closely. When the blood sugar concentration was high, over 300 mg. of sugar per hundred cubic centimeters of blood, at the onset of the work, it required at least a week of low blood sugar, 160 mg. or less, before Q-R-S remained at a consistently low level. This low level was not permanent, for the height of the main deflection increased gradually with an increase in the sugar content. If the blood sugar concentration was again reduced, the fall to the original low level occurred in a much shorter time than in the beginning (Table 2).

In all of the cases with very high blood sugar concentrations, there was notching of the main deflection. Table 3 is illustrative of the findings in this group.

The same results were obtained in these six cases as in the previous cases, namely, the higher the sugar concentration of the blood at the time the tracing was taken, the higher the main deflection.

ANIMAL STUDIES

This work on human diabetic patients was corroborated by animal experimentation. Dogs were used, and the blood sugar concentration was increased by various means. Electrocardiograms were taken at regular intervals and carefully standardized. In each case, an electrocardiogram was taken as soon as the animal was quiet, and blood was immediately drawn from the jugular vein for sugar estimation. The animal was then anesthetized with ether, and an electrocardiogram taken as soon as relaxation was complete. Blood was drawn from the right jugular vein as soon as the last lead had been taken. It is a well known fact that the percentage of blood sugar rises during anesthesia. The

TABLE 2.—*Changes in Deflection Associated with Changes in Blood Sugar Concentration in Same Subject*

Case*	Date	Sugar	R. Lead I	R. Lead II	R. Lead III	Tracing
1	2/10/25	262 mg.	13 mm.	8 mm.	10 mm.	1
	2/16/25	190 mg.	11 mm.	7 mm.	9 mm.	3
2	2/10/25	242 mg.	8 mm.	9 mm	5 mm.	2
	2/14/25	320 mg.	9 mm.	9 mm.	6 mm.	5
	2/18/25	177 mg.	8 mm.	8 mm.	5 mm.	8
	2/22/25	230 mg.	10 mm.	7 mm.	5 mm.	9

* Case 1, a man, aged 61; Case 2, a man, aged 53.

TABLE 3.—*Notching of Main Deflection Associated with High Blood Sugar Concentration* *

Date	Sugar	R. Lead I	R. Lead II	R. Lead III	Tracing
2/11/25	308 mg.	14 mm.	4 mm. (notched)	2 mm. (notched)	1
2/16/25	330 mg.	11 mm.	5 mm. (notched)	2 mm. (notched)	5
2/22/25	252 mg.	8 mm.	4 mm. (notched)	2 mm. (notched)	6

* The patient, a young man, aged 17, had been under observation for three years, and received 60 units of insulin daily.

work of Banting and Best and their co-workers [6] corroborates this statement. The latter found that the rise of blood sugar in rabbits is rapid and persists as long as etherization continues. The same findings held good in our work on dogs. There was a marked initial rise of blood sugar, on an average from 92 to 150 mg. of sugar per hundred cubic centimeters within twenty minutes after anesthetization, then a continual fairly steady rise for the five or six hours that the experiment was continued. There was a marked initial rise of the main deflection of the string with the beginning of anesthetization, but subsequent tracings showed only slight alteration.

6. Banting, F. G.; Best, C. H.; Collip, J. B.; MacLeod, J. J. R., and Noble, E. C.: The Effects of Insulin on Experimental Hyperglycemia, Am. J. Physiol. **62**:559 (Nov.) 1922.

A second group of animals received 500 c.c. of a 10 per cent. solution of glucose intravenously into the right femoral vein. The dogs were kept under the influence of ether throughout the course of the experiment and electrocardiographic tracings and blood sugar estimations were made every hour. In this group (Table 4) as in the preceding, there was a sudden sharp rise of the Q-R-S deflections as soon as the animal was anesthetized. There was a marked increase in the blood sugar concentration as the intravenous solution was continued. There was a slight increase in the swing of the galvanometer string, but this was not as great as had been expected.

A third series of animals received Ringer's solution intravenously in the place of glucose solution. In this group there was the initial increase in height of the main deflection, with etherization, then a slight fall in one case, or constant findings in spite of the fact that there was an increase in the sugar content.

TABLE 4.—*Protocols of Two Typical Experiments on Dogs, Illustrating Changes Associated with Increased Blood Sugar Content Due to Ether Anesthesia and Following Intravenous Glucose Injection*

Animal	Blood Sugar	R. Lead I	R. Lead II	R. Lead III	
1	77 mg.	8 mm.	22 mm.	18 mm.	Before anesthesia
	130 mg.	9 mm.	21.5 mm.	18.5 mm.	After anesthesia
	502 mg.	5 mm. (notched)	23 mm.	21 mm.	After 25 gm. of glucose
	680 mg.	6.5 mm. (notched)	24 mm.	25 mm.	After 45 gm. of glucose
2	97 mg.	14 mm.	13 mm.	15 mm.	Before anesthesia
	180 mg.	15 mm.	19 mm.	8 mm. (slur)	After anesthesia
	294 mg.	17 mm.	11 mm.	6 mm. (notched)	After 25 gm. of glucose
	480 mg.	14 mm.	20 mm.	10 mm. (notched)	After 45 gm. of glucose

The last group of animals received insulin following the initial tracings under normal conditions and after preliminary etherization. It was very difficult to overcome the hyperglycemia due to ether anesthesia. As Banting, Best and their co-workers [7] have shown, there is a latent period during anesthesia before insulin acts. When less than 1 unit of insulin was given intravenously every fifteen minutes, the blood sugar at the end of three hours remained higher than before ether was begun. When more than this amount was used the animal died within an hour or so. These findings are in accord with those of Bliss.[8] He states that a dog weighing 9 kg. can stand six units of insulin daily, the entire amount within a few hours producing a stormy result. When one unit every four hours was given, fairly good results were obtained by him.

7. Banting, F. G.; Best, C. H.; Collip, J. B.; MacLeod, J. J. R., and Noble E. C.: Effects of Pancreatic Extract (Insulin) on Normal Rabbits, Am. J. Physiol. **62**:162 (Sept.) 1922.

8. Bliss, S. W.: The Effects of Insulin on Diabetic Dogs, J. Metab. Res. **2**:385 (Sept.) 1922.

COMMENT

Electrocardiographic tracings of diabetic patients showed notching of the main deflections more often than tracings of other subjects of the same age and sex. The higher the blood sugar concentration, the more frequent the notching, and as the sugar concentration decreased the notching tended to disappear. This notching was not due to a bundle branch lesion because such cases have, according to Lewis,[9] the following features in common:

1. The amplitude of the chief deflections are more than normal. 2. The initial phases have an unusual duration. 3. The final deflection is opposite in direction to the chief initial deflection, R or S, as the case may be.

None of the tracings included showed any of the three features mentioned above. As yet we are uncertain in what pathologic conditions notching of R occurs with no delay, but it is generally believed that myocardial changes are present in these cases. Since there was a reduction in the notching with decreased sugar content, it seemed probable that there was an improvement of the myocardial condition. It required several weeks of low sugar concentration to cause any marked change in the notching.

The height of the main deflection of the electrocardiographic tracing was greater in diabetic patients than in persons in the control groups of the same age and sex. As the sugar content of the circulating blood increased, there was an increase in the height of Q-R-S in the electrocardiograms, while a decrease in the sugar content caused a smaller swing of the galvanometer string. The sugar content of the circulating blood seemed to influence the action current of the cardiac muscle. These changes can be measured quantitatively, and seem to parallel the quantity of blood sugar fairly exactly. This is in accord with the work of Belehradek and Noyons,[5] but in our patients the changes were less extreme than in their work on perfused animal hearts. This was to be expected because changes in sugar concentration in human blood can never be so sudden or so marked as in perfused animal hearts. In the case of human hearts, outside factors could not be regulated nor excluded as compared with the regulation of perfused hearts. Within the limit of experimental error, it seemed certain that some change occurred in the electrocardiographic tracing with changes of sugar concentration.

It was not only Q-R-S that showed changes in amplitude with changes in blood sugar concentration. The P wave (due to auricular activity) also showed definite changes in height of excursion. This

9. Lewis, T.: The Mechanism and Graphic Registration of the Heart Beat, London, Shaw and Sons, 1920, p. 120.

was much more difficult to measure quantitatively under the present conditions of standardization. From the very insufficient evidence at hand, it seemed that P changed in the same way as the main deflection changed with blood sugar changes.

Following ether anesthetization, dogs showed a marked and fairly constant hyperglycemia of about the same severity as rabbits show under the same circumstances. The electrocardiograms showed an increase in the height of the main deflection as soon as the dog was under the influence of the ether and relaxation was complete. This rise remained fairly constant as long as the ether was continued, and during this period other factors had little influence on the resulting tracing. When intravenous sugar solutions were given for several hours, there was very little change on the swing of the galvanometer string, after the initial rise due to etherization. Beyond a certain sugar concentration no change could be detected, even when many times the amount of sugar was given which was originally present. When physiologic sodium chlorid solution was given, it was never possible to give enough fluid to overcome the increased blood sugar due to ether. When insulin was used, there was likewise no change because enough insulin to overcome the effects of etherization always caused death within a short time.

Since changes in blood sugar concentration seemed to produce definite changes in the action current, it is suggested that the sugar acts directly on the cardiac muscle. This was more marked when perfused hearts were employed than when human hearts were used. The changes that occurred were more marked when the solution contained a large amount of sugar, but this was not a definitely quantitative thing. The results obtained make it appear possible that sugar in high concentrations may be considered a protoplasmic poison, and acts in a toxic manner on the cardiac muscle. As yet sufficient evidence is lacking for a positive statement on this point.

SUMMARY

Notching of the main deflection of the electrocardiographic curves occurred more frequently in curves of diabetic patients than in curves of control patients.

Changes in the blood sugar concentration were associated with changes in the main deflections of the electrocardiographic tracings.

Increasing the amount of blood sugar in dogs by etherization or by glucose injections caused an increase in the height of the Q-R-S wave, while a decrease in the amount of blood sugar produced a decrease in the amplitude of the Q-R-S wave.

NONSPECIFIC DESENSITIZATION THERAPY IN ALLERGIC ASTHMA

THE EOSINOPHILIC INDEX AS A GUIDE TO INTRAMUSCULAR INJECTION OF VENOM PROTEIN, WITH CASE REPORT

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Nonspecific desensitization and nonspecific protein therapy have become recognized factors in immunity. Rosenau and Anderson [1] have shown that animals receiving repeated injections of foreign proteins become markedly insusceptible to anaphylactic shock. Longcope [2] writes: "It seems impossible to avoid the conclusion that in animals sensitized to two proteins anaphylactic shock to the one reduces temporarily the sensitiveness to the second." Dale [3] found that a guinea-pig sensitized to several serums and also to egg white could be desensitized to the serum with egg white. Kolmer,[4] in referring to nonspecific protein therapy, says that "nonspecific activities of bacterial vaccine may exert beneficial and curative effects in disease to which the bacteria incorporated in the vaccine has no etiologic relationship."

CLINICAL REPORTS ON NONSPECIFIC DESENSITIZATION IN ASTHMA

Numerous clinical reports are accumulating which show the beneficial effect of various nonspecific desensitizing agents in asthma as well as in hay-fever, and other sensitization conditions. Miller [5] gave intravenous injections of typhoid vaccine in a number of cases of bacterial asthma with relief to some of the patients. Those not benefited were treated with autogenous vaccine. He says: "In no case, however, that had failed to be relieved by the typhoid vaccine was there any benefit from autogenous vaccine." Auld [6] has used peptone intravenously in the treatment of asthma. Recently, he has reported on the beneficial use of peptone and serum in eleven cases. Miller,[7] in a recent article, concludes: "Peptone therapy in asthma and allied conditions offers a simple and effective mode of treatment." Veitch [8] treated twenty-four

1. Rosenau and Anderson, quoted by Miller, J. L.: Am. J. M. Sc. **168**:639 (Nov.) 1924.
2. Longcope, W. T.: Physiological Rev. **3**:290, 1923.
3. Dale, H. H.: Brit. M. J. **2**:689 (Oct. 29) 1921; ibid. **1**:45 (Jan. 12) 1922.
4. Kolmer, J. A.: Infection, Immunity and Biological Therapy, Ed. 3, Philadelphia, W. B. Saunders Company.
5. Miller (Footnote 1).
6. Auld, A. G.: Brit. M. J. **1**:696 (May 14) 1921; ibid., March 7, 1925.
7. Miller, H. P.: Illinois M. J. **45**:269 (April) 1924.
8. Veitch, John: Brit. M. J. **1**:13 (Jan. 5) 1924.

asthmatic patients with weekly intramuscular injections of peptone combined with a mixed catarrheal vaccine. He reports fourteen patients cured; four greatly improved; five improved to a less degree, and one not improved. Van Leeuwen and Varekamp [9] treated twenty-eight patients with bacterial bronchial asthma who gave positive Pirquet and negative skin tests, with Koch's tuberculin. With this form of nonspecific desensitization they report 68 per cent. of the patients completely relieved, 10 per cent. greatly benefited, 18 per cent. decidedly improved and 4 per cent. not benefited. The parenteral injection of milk in asthma has been reported of benefit by Schiff [10] and other clinicians.

In a recent article,[11] I reported beneficial results in sensitization diseases, including asthma, hay-fever, migraine and epilepsy from the intramuscular injection of venom protein (crotalin) solution, which contains a peptone and a globulin.

EOSINOPHILIA IN ASTHMA

The local collection of eosinophil cells in the lungs in asthma is a clinical observation that has been frequently recorded in textbooks. The almost invariable finding of an eosinophilia in the peripheral blood at some stage of an asthmatic attack, provided the differential leukocyte counts are made at repeated intervals, is very suggestive that the underlying factor, in all cases of true bronchial asthma, is of allergic origin. This increase of eosinophil cells demonstrable before, during or soon after an asthmatic paroxysm is highly significant of the relation of asthmatic hypersensitivity to anaphylactic or anaphylactoid phenomena. The asthmagenic substance to which a patient is sensitized is undoubtedly positively chemotactic for eosinophil cells. Van Leeuwen and Leydner [12] report isolating a muscle stimulating substance from the blood of patients with asthma, urticaria, migraine and epilepsy, all diseases in which an increase of eosinophil cells has been noted. They could not demonstrate its presence in other diseases or in normal subjects. They believe that this toxic substance is related to the "allergic disposition" of the patient.

EOSINOPHILIA IN THE ANAPHYLACTIC-LIKE REACTION FOLLOWING VENOM PROTEIN INJECTION

Investigators are pointing out that commonly an increase in the percentage of eosinophil elements in the differential blood count is pres-

9. Van Leeuwen, W. S., and Varekamp, H.: Lancet **1**:201, 1921; ibid. **2**:1366 (Dec. 31) 1921.
10. Schiff, N.: Am. J. M. Sc. **166**:664 (Nov.) 1923.
11. Spangler, R. H.: M. J. & Rec. **122**:313 (Sept. 16) 1925.
12. Van Leeuwen, W. S., and Leydner: Brit. J. Exper. Path. **3**:282 (Dec.) 1922.

ent during the anaphylactic-like phenomena that follow the parenteral injection of foreign protein substances.

In a number of articles [13] published since 1912, I have called attention to the fact that the mechanism of the reaction following the intramuscular injection of venom protein (crotalin) solution includes an increase in the percentage of eosinophil cells in the differential count; this is in addition to the lengthening of the clotting of the blood, the increasing of the permeability of the vessel walls and the producing of general cell and glandular stimulation, all recognized factors in the mechanism of the nonspecific protein reaction. Schlecht's [14] experiments on guinea-pigs and dogs, showing that with repeated small injections of various foreign protein substances an eosinophilia is produced, confirm my findings.

Since eosinophils are found increased in anaphylaxis and anaphylactic-like conditions it would seem plausible that an estimate of their varying percentage would be of value in determining the degree of the nonspecific reaction, when it is used as a therapeutic measure.

SUGGESTED METHOD FOR REGULATING STRENGTH OF DOSE AND FREQUENCY OF ADMINISTRATION OF NONSPECIFIC PROTEIN AGENTS

It would be of the greatest value if a method could be established for regulating the strength of dose and the frequency with which a protein injection should be given in order to keep a patient nonspecifically desensitized. The biologic test of giving an injection and depending on the resulting clinical manifestations is most unsatisfactory and often misleading.

In an experience with intramuscular injections of venom protein (crotalin) solution covering a period of fifteen years, during which time more than 16,000 intramuscular injections have been given to over 700 patients, I have found that a study of the differential blood count before and after injections with a special reference to the degree of eosinophilia produced (eosinophilic index) offers a satisfactory guide to both strength of dosage and frequency of administration of the protein substance. I have reported this method in detail in previous articles,[15] and in submitting it at this time as a practical guide for the administration of nonspecific desensitization therapy in asthma I will summarize my findings briefly.

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13. Spangler, R. H.: New York M. J., Oct. 4, 1913; ibid. **104**:534 (Sept. 16) 1916; ibid. **107**:727 (April 20) 1918.

14. Schlecht: Deutsch. Arch. f. klin. Med. **108**:405, 1912.

15. Spangler, R. H.: M. J. & Rec. **120**:495 (Nov. 19) 1924; Atlantic M. J. **28**:138 (Dec.) 1924.

EOSINOPHILIC INDEX A GUIDE

As a rule the highest rise in the percentage of eosinophils, following venom protein injections, which I have used in the range of doses $\frac{1}{400}$ to $\frac{1}{50}$ grain, occurs by the second or third day. In from five to seven days after injection, the eosinophil cells will, as a rule, have dropped to 4 per cent. or under (unless a higher percentage range of eosinophils had been present in the patient before the first injection was given), and the patient may be given another injection. It has been my practice not to repeat an injection if the percentage of eosinophil cells by the fifth day has not dropped to within the normal range, or at least as low as they were before starting the protein injections. Moreover, it has not seemed wise to increase the strength of the dose if any given strength is producing an increase of from 8 to 10 per cent. of eosinophils by the second or third day after an injection.

The following case report shows the clinical results of nonspecific protein desensitization following repeated intramuscular injections of venom protein (crotalin) solution in a case of bronchial asthma and hay-fever. The accompanying table and chart illustrate the value and significance of recording the eosinophilic index.

REPORT OF CASE

A woman, aged 30, a librarian, who had been suffering with severe asthmatic paroxysms for three years, came under my care, April 11, 1924. Her mother had been subject to nocturnal convulsions at irregular intervals from her eighteenth to her twenty-fifth year. At that age the convulsions stopped and she developed migraine seizures with vomiting, which occurred periodically and persisted until the menopause. A maternal aunt also had migraine.

The patient was the youngest of four children, had been a full term baby and had had a normal birth. She was bottle fed and suffered much with digestive disturbances before teething. As a girl she had "bilious attacks" and frequently suffered with hives. At the twelfth year she began to have migraine, with vomiting. Menstruation was established at 13 years; she was always regular and had little pain. Two months later, in August, 1908, she developed hay-fever, which has recurred periodically each autumn since (sixteen years) in spite of pollen therapy, change of climate, rhinologic treatments, the use of calcium, parathyroid and various other drugs.

Just before the annual hay-fever season of 1921 she developed acute asthmatic paroxysms; at first these were mostly nocturnal but during the year before I saw her they were occurring both day and night, and the patient required epinephrin or morphin hypodermically almost daily. It was necessary for the patient to give up her library work. Food skin tests were negative. Skin reactions to feathers and house dust were positive but the avoidance or removal of these irritants to the best of her ability gave no relief. The physical signs in the case were typical of an acute bronchial asthmatic paroxysm. A urine examination was negative. The blood Wassermann test was negative.

April 11, the hemoglobin was 78 per cent.; the red cells 3,950,000, and the white cells 7,200. The differential counts on four days out of the next five, during which time she had five severe and four medium asthmatic paroxysms, showed respectively a 9-, 15-, 11- and 14 per cent. of eosinophil cells.

April 16, a $\frac{1}{600}$ grain of crotalin was given intramuscularly, followed by a very mild local reaction. The percentage of eosinophils two days later was 11, and there were four light asthmatic attacks.

April 21, a second 1/600 grain of crotalin was given in the other arm. This was followed by a marked local reaction and two days later the eosinophil cells were 21 per cent. She had had two severe paroxysms, April 20. The percentage of eosinophilia, April 25, was 16, August 26, 11.

April 28, the eosinophils were 9 per cent. and I gave a third 1/600 grain injection of crotalin solution, with another marked local reaction. She had two severe paroxysms, April 29, and one in my office, April 30. There had been, however, an interval of nine days between seizures (the longest freedom from attacks for several months). The eosinophils, April 30, were 16 per cent. May 1, there was a hard paroxysm that lasted several hours. The eosinophils that day were 14 per cent.

May 5, the eosinophils were 10 per cent. and I gave a 1/600 grain of crotalin, which was followed by a good local reaction. Two days later, May 7, the eosinophils were 15 per cent. There had again been an interval of six days without an asthmatic paroxysm when, during the night of May 7, a severe attack occurred.

May 12, the eosinophils were 8 per cent. in the morning and I gave a 1/600 injection of crotalin. That night the patient had a very severe and long asthmatic seizure, which lasted nearly four hours. Two days later, May 14, the eosinophils were 13 per cent.

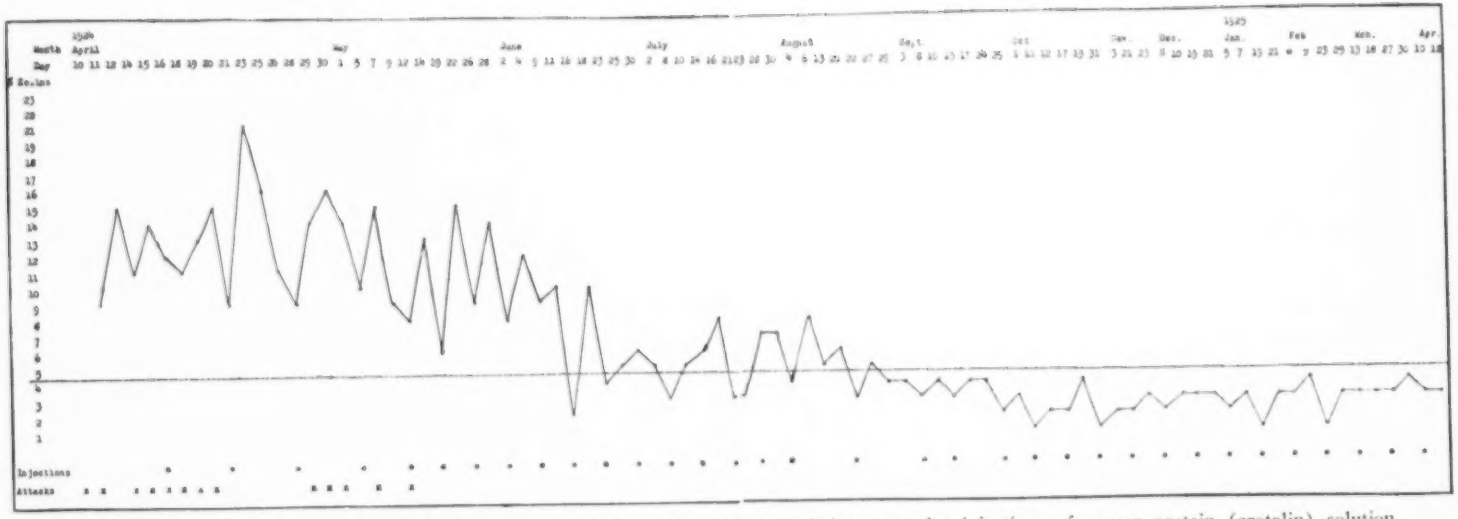
COMMENT ON TABLE AND CHART

By referring to the table and chart, it will be noted that for five days before any protein injections were given and during which time the patient was having repeated acute asthmatic paroxysms, the percentage of eosinophil cells ranged from 9 to 15. The total white count was 6,800. This patient was undoubtedly sensitized to some undetermined asthmogenic substance.

The first crotalin injection, April 16, produced only slight local reaction and no change in the percentage of eosinophils. The second injection, however, five days later (April 21) was followed by a severe local reaction, which lasted forty-eight hours. It was accompanied by a slight leukopenia; the total white count dropped to 4,200. The eosinophil cells, forty-eight hours after the injection, were 21 per cent. There evidently had been superposed a nonspecific sensitization with the production of an anaphylactic-like state as indicated by the leukopenia and the decided increase in the percentage of eosinophil cells. It is of clinical interest that no asthmatic seizures occurred for nine days following this anaphylactic-like phenomenon.

Crotalin injections were then continued at seven day intervals and, as indicated by the accompanying chart and table, were followed each time by an increase of eosinophils; but the percentage range of eosinophils gradually decreased and by June 16, eight weeks after the first crotalin injection, they dropped to within the normal range (4 per cent. or under).

During the next two months the percentage of eosinophils showed moderate increase following the crotalin injections, but they never went



Eosinophilic index in a case of allergic asthma nonspecifically desensitized with intramuscular injections of venom protein (crotalin) solution.

Data in Case of Woman Asthmatic Patient, Aged 30, Nonspecifically Desensitized with Intramuscular Injections of Venom Protein (Crotalin) Solution

Date	Dose of Crotalin, Grains	Local Reaction	Polymorphonuclears, per Cent.	Differential Leukocyte Count					Attacks
				Small Lymphocytes	Large Lymphocytes	Eosinophils	Basophils	Transitionals	
1924									
4/11	.....	.....	60	24	4	9	—	3	1 severe, 1 medium
4/12	.....	.....	59	16	9	15	1	—	3 medium
4/14	.....	.....	58	28	2	11	—	1	1 severe, 2 medium, 1 severe
4/15	.....	.....	54	23	3	14	1	5	1 severe, 2 medium, 2 mild
4/16	.....	1/600	Slight	50	32	4	12	—	2	1 medium
4/18	.....	.....	.....	52	31	4	11	1	1	4 mild
4/19	.....	.....	.....	51	25	6	13	1	4	1 severe
4/20	.....	.....	.....	54	18	8	15	2	3	2 severe
4/21	.....	1/600	Marked	60	23	5	9	—	3	
4/23	.....	.....	.....	49	19	6	21	1	4	
4/25	.....	.....	.....	54	25	3	16	1	1	
4/26	.....	.....	.....	57	22	5	11	—	5	
4/28	.....	1/600	Marked	51	31	5	9	2	3	
4/29	.....	.....	.....	52	25	4	14	1	4	2 severe
4/30	.....	.....	.....	53	26	2	16	—	3	1 severe
5/ 1	.....	.....	.....	48	24	7	14	2	5	1 severe
5/ 5	.....	1/600	Good	50	30	3	10	1	6	
5/ 7	.....	.....	.....	49	27	4	15	—	5	1 severe
5/ 9	.....	.....	.....	54	29	4	9	1	3	
5/12	.....	1/600	Moderate	52	30	5	8	—	5	1 severe
5/14	.....	.....	.....	49	27	5	13	1	5	
5/19	.....	1/600	Very fair	58	27	7	6	—	2	
5/22	.....	.....	.....	59	14	9	15	1	2	
5/26	.....	1/600	Very fair	51	32	4	9	—	4	
5/28	.....	.....	.....	42	36	6	14	—	2	
6/ 2	.....	1/600	Fair	56	28	3	8	1	4	
6/ 4	.....	.....	.....	50	31	4	12	—	3	
6/ 9	.....	1/600	Fair	45	35	7	8	—	5	
6/11	.....	.....	.....	45	30	8	10	2	5	
6/16	.....	1/600	Fair	52	41	3	2	—	2	
6/18	.....	.....	.....	50	30	6	10	1	3	
6/23	.....	1/600	Slight	56	30	3	4	1	3	
6/25	.....	.....	.....	46	38	6	5	1	4	
6/30	.....	1/600	Slight	45	35	9	6	—	5	
7/ 2	.....	.....	.....	48	34	6	5	1	6	
7/ 8	.....	1/400	Fair	55	30	7	3	—	5	
7/10	.....	.....	.....	50	32	8	5	2	3	
7/14	.....	1/400	Fair	48	35	7	6	1	3	
7/16	.....	.....	.....	43	40	4	8	1	4	
7/21	.....	1/400	Fair	50	38	6	3	—	3	
7/23	.....	.....	.....	48	40	7	3	—	2	
7/28	.....	1/400	Fair	48	35	9	7	—	1	
7/30	.....	.....	.....	45	39	6	7	1	3	
8/ 4	.....	1/400	Fair	48	37	7	4	1	3	
8/ 6	.....	.....	.....	50	31	6	8	2	3	
8/13	.....	.....	.....	48	33	7	5	2	5	
8/20	.....	.....	.....	52	31	5	6	1	5	
8/22	.....	1/400	Slight	56	32	6	3	—	3	
8/27	.....	.....	.....	49	34	8	5	1	3	
8/29	.....	1/400	Slight	48	36	8	4	—	3	
9/ 3	.....	.....	.....	50	34	7	4	1	4	
9/ 8	.....	1/400	Slight	50	38	7	3	—	2	
9/10	.....	.....	.....	52	35	6	4	—	3	
9/15	.....	1/400	Slight	50	35	7	3	1	4	
9/17	.....	.....	.....	45	40	7	4	1	3	
9/24	.....	.....	.....	48	37	6	4	2	3	
9/29	.....	1/400	Slight	54	32	6	2	1	5	
10/ 1	.....	.....	.....	50	36	8	3	—	3	
10/10	.....	1/400	Slight	50	38	5	1	2	4	
10/12	.....	.....	.....	48	40	6	2	1	3	
10/17	.....	1/400	Slight	58	32	7	2	—	6	
10/19	.....	.....	.....	52	31	8	4	—	5	
10/31	.....	1/400	Slight	54	35	9	1	1	2	
11/ 3	.....	.....	.....	50	34	8	2	1	5	
11/21	.....	1/400	Slight	52	36	9	2	1	3	
11/23	.....	.....	.....	50	37	6	3	—	4	
12/ 8	.....	1/400	Slight	52	38	7	2	—	1	
12/10	.....	.....	.....	55	34	5	3	1	2	
12/19	.....	1/200	Fair	58	30	7	3	—	2	
12/21	.....	.....	.....	57	29	9	3	—	2	
1925									
1/ 5	.....	1/200	Fair	55	35	8	2	—	—	
1/ 7	.....	.....	.....	53	37	7	3	—	—	
1/19	.....	1/200	Slight	59	34	5	1	—	1	
1/21	.....	.....	.....	55	32	7	3	1	2	

higher than 8 per cent. and as a rule each week dropped to within the normal range. Since Aug. 22, 1924, and up to the present time, a period of eight months, during which crotalin injections have been given at two or three week intervals, the percentage of eosinophils, as shown on the chart, has never risen above the normal line.

If my interpretation of the eosinophilic index is correct these facts would indicate immunologically that a state of antianaphylaxis has been maintained and that the patient has remained, as I would term it, nonspecifically desensitized.

CLINICAL RESULT

Clinically, it is very interesting to note that the last asthmatic seizure was the severe paroxysm which the patient had on the night of May 12, 1924. Since that time (eleven months) the patient has remained entirely free from all asthmatic symptoms. Moreover, she did not develop any hay-fever symptoms during August and September, 1924, the first season she had missed for sixteen years. Since Sept. 1, 1924, this patient has been regularly at her library work and reports that she is "perfectly well and the enemy is conquered." [16]

Data in Case of Woman Asthmatic Patient, Aged 30, Nonspecifically Desensitized with Intramuscular Injections of Venom Protein (Crotalin) Solution—Continued

Date	Dose of Crotalin, Grains	Local Reaction	Polymorphonuclears, Per Cent.	Differential Leukocyte Count					Attacks
				Small Lymphocytes	Large Lymphocytes	Eosinophils	Basophils	Transitionals	
2/ 6	1/200	Slight	58	30	8	3	—	1	
2/ 9	.....	.....	58	33	5	4	—	—	
2/23	1/200	Slight	57	32	8	1	—	2	
2/25	.....	.....	55	28	7	3	1	2	
3/13	1/200	Slight	55	27	5	3	—	1	
3/18	.....	.....	58	32	7	3	—	—	
3/27	1/200	Slight	56	33	8	3	—	2	
3/30	.....	.....	53	35	8	4	—	—	
4/10	1/200	Slight	56	33	7	3	—	2	
4/12	.....	.....	54	34	6	3	—	3	

CONCLUSIONS

1. Nonspecific desensitization therapy has become a recognized factor in immunity.

2. Various nonspecific protein agents have been found of clinical value in the treatment of allergic asthma and may at times be of benefit even when specific serums and vaccines have given no relief.

3. The increase of eosinophils in the differential blood count in true bronchial asthma points significantly to the presence of an allergic factor in the affliction.

16. Since this report was written the 1925 season has passed and the patient has remained free of hay-fever symptoms.

4. The varying precentage of eosinophil cells (the eosinophilic index) has been found a valuable guide in regulating the strength of dose and frequency of administration of venom protein when it has been used as a nonspecific desensitizing agent in allergic asthma.

THE NATURE OF SO-CALLED SINO-AURICULAR BLOCK *

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Sino-auricular block is a term applied to a relatively uncommon arrhythmia in which, in an otherwise regular rhythm, dropped beats occur, both auricular and ventricular beats participating in the intermittence. This definition is not strictly true because sino-auricular block is frequently associated with sinus arrhythmia. The long pause is not always equal to exactly two normal cycles, being sometimes shorter, at other times longer. Occasionally, too, the long cycle is preceded by a quickening of the auricular rate and is followed by cycles that are of somewhat greater duration than the average cycles of the normal rhythm.

For a discussion of the literature on the subject of sino-auricular block, reference may be made to Lewis,[1] and also to the papers of Levine [2] and Eyster and Evans.[3] Most of the articles are concerned with reports of clinical cases. In them the view has been generally accepted that the arrhythmia is due to a block between the sino-auricular node and the auricle. This assumption is based on the fact that a block between sinus venosus and auricle can be produced in the cold blooded heart when a ligature is placed below the sinus; and also because the arrangement of beats, which frequently, but not always, takes place at the time the intermittence of the auricle occurs, is very much like that seen in auriculoventricular block when a ventricular beat drops out. Lewis [1] has pointed out, however, that there is no clear anatomic or physiologic basis for the acceptance of this view.

The only experimental work that shows definitely that the arrhythmia that is being discussed may actually occur is that of Eyster and Meek.[4] In one experiment, out of a number, in which the sino-auricular node was separated from the rest of the heart on three sides by ligatures and a clamp, clamping the fourth side produced a condition in which the

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1. Lewis, T.: The Mechanism and Graphic Representation of the Heart Beat, London, Shaw and Sons, 1920.

2. Levine, S. A.: Observations on Sino-Auricular Block, Arch. Int. Med. **17**:153 (Jan.) 1916.

3. Eyster, J. A. E., and Evans, J. S.: Sino-Auricular Heart Block, with Report of a Case in Man, Arch. Int. Med. **16**:832 (Nov.) 1915.

4. Eyster, J. A. E., and Meek, W. J.: Experiments on the Origin and Conduction of the Cardiac Impulse, VI, Conduction of the Excitation from the Sino-Auricular Node to the Right Auricle and Auriculo-Ventricular Node, Arch. Int. Med. **18**:775 (Dec.) 1916.

transmission interval between the sino-auricular node and the auriculoventricular node increased progressively until each fifth or sixth impulse failed to reach the auriculoventricular node. This was proved by placing electrodes over the sino-auricular and auriculoventricular nodes, each pair being connected with an electrocardiograph. In two other experiments, by completely isolating the sino-auricular node they were able to obtain complete sino-auricular block, the rhythmic rate of the sino-auricular node being greater than that of the rest of the heart.

These experiments are hardly applicable to the condition as it is seen clinically. In the first place, there is no evidence that clinically, when so-called sino-auricular block occurs, the sinus node is completely surrounded by diseased tissue, incapable of transmitting impulses arising in the sinus. In a large percentage of the cases in which sino-auricular block has been present, the arrhythmia has been transient and has resulted apparently from the administration of digitalis;[2] in other cases it has been present in apparently healthy individuals, of which one, reported by Eyster and Evans,[3] may be cited. However, since sino-auricular block is in most instances dependent on vagal action, as it is frequently associated with other evidences of vagal activity and practically always disappears under atropin, and since it may be produced experimentally by drugs that stimulate the vagus nerves,[5] it is possible that the block between sinus and auricle may be due to vagal tone, just as auriculoventricular block may be occasioned by vagal stimulation. In other words, whereas in Eyster and Meek's experiments a block between sinus and auricle was induced by clamping, clinically a comparable block may be due to vagal tone. This argument, too, is invalid when one attempts to draw an analogy between the experiments of Eyster and Meek, and the condition as it is seen clinically. It has been shown by Lewis and Drury[6] and more recently by Drury[7] that when impulses from one part of the auricle are impeded in their transmission to another by clamping, vagal stimulation *decreases* the block rather than increases it. Whether this is true exactly at the sino-auricular junction is not known. But in the experiments of Eyster and Meek the clamp was placed a few millimeters from the border of the sinus node on auricular tissue. One cannot, then, draw inferences as to the clinical condition from these experiments. All that one can say is that they demonstrate the possibility of the occurrence of clinical sino-auricular

5. Cushny, A. R.: The Irregularities of the Mammalian Heart Observed Under Aconitin and on Electrical Stimulation, Heart **1**:1, 1909-1910. Eyster, J. A. E., and Meek, W. J.: Cardiac Irregularities in Morphin Poisoning in the Dog, Heart **4**:59, 1912-1913.

6. Lewis, T., and Drury, A. N.: The Effect of Vagal Stimulation on Intra-Auricular Block Produced by Pressure or Cooling, Heart **10**:179 (April) 1923.

7. Drury, A. N.: Further Observations Upon Intra-Auricular Block Produced by Pressure or Cooling, Heart **12**:143, 1925.

block due to a lesion similar to the one they have produced experimentally, but that most clinical cases probably do not fall into this group. Barker and Kinsella [8] have described sino-auricular block in a dog the subject of experimental streptococcus endocarditis. On histologic examination it was found that the sinus node, practically normal itself, was completely surrounded by diseased tissue. However, they are clear in stating that their observation is not applicable to the explanation of most instances of clinical sino-auricular block.

In former experiments [9] it has been shown that following the intravenous injection of pituitary extract into normal unanesthetized dogs, there developed an arrhythmia which is similar to the one termed sino-auricular block, dependent on central stimulation of the vagus nerves. As the effect of the pituitary extract wore off, sinus arrhythmia appeared. Since there was no sharp transition between the two types of irregularity, the one merging gradually into the other, it was suggestive that there was an intimate relationship between the two. The sino-auricular block seen in these experiments seemed to be comparable to that which one observes clinically, and an opportunity was offered of investigating this arrhythmia further. By placing electrodes directly over the sino-auricular node, at the same time using one of the usual leads, and recording the activity of the sinus and of the auricle as a whole simultaneously, it appeared possible to determine whether or not the irregularity was due to a block between the sinus and the auricle. If a block actually exists, one should obtain on the same record evidence of sinus activity at the time the auricular beat drops out. On the other hand, if the intermittence is due to a failure of the sinus to generate an impulse, then there should be an absence of activity of both the sinus and the auricle as a whole.

EXPERIMENTAL METHODS

Six experiments were done, only three of which are described below. The other three are discarded because after the chest was opened the injection of pituitary extract had practically no effect except to slow the rate of the heart. In the three that are reported below, medium size dogs were used. They were anesthetized by the subcutaneous injection of morphin (or pantopon), followed within fifteen minutes by the administration of urethan by stomach tube. The exact amounts of the drugs are given in the protocols. In order to minimize the disturbances that might result from the operative procedure, 0.5 per cent. procain solution was used locally. A part of the fourth right rib was resected

8. Barker, P. S., and Kinsella, R. A.: Sino-Auricular Block in a Dog Suffering from Experimental Streptococcus Endocarditis, Heart **11**:81, 1923.

9. Resnik, W. H., and Geiling, E. M. K.: The Action of Pituitary Extract on the Heart of the Unanesthetized Dog, J. Clin. Invest. **1**:217 (Feb.) 1925.

under artificial respiration (intratracheal insufflation), this affording ample exposure of the heart. A small incision was made in the pericardium over the sulcus terminalis, and woolen threads moistened with physiologic sodium chlorid solution were attached to the surface of the heart, the upper one just below the cavo-appendicular junction, the lower one a few millimeters below this, both on the sulcus terminalis. These electrodes were situated, then, over the normal pacemaker of the heart.[10] The woolen threads were attached to the heart by very fine silk ligatures placed in the epicardium, the area of contact with the surface of the heart being about 2 mm. in diameter. The woolen threads were then insulated by leading them through small glass tubes which extended from the surface of the heart (particular care being used to see that these ends of the glass tubes were smooth and rounded) and out through the chest wall. The wound in the chest wall was then closed after the air in the pleural cavity was evacuated, respiration then being carried on automatically, a small stream of oxygen flowing through the intratracheal cannula. The protruding ends of the woolen threads were then inserted into kaolin plugged glass tubes filled with a saturated solution of copper sulphate in which were the ends of the wires leading to one galvanometer, which now recorded the electrical activity of the sino-auricular node. To another galvanometer were connected small German silver electrodes sewn under the skin of the right upper and left lower limbs (Lead II), this galvanometer recording the activity of the auricles as a whole, and, of course, of the ventricles. Both records were taken simultaneously on the same film.

After several records of the normal heart's action were taken, from 0.5 to 1 c.c. of pituitary extract (Armour's Pituitary Liquid) was injected intravenously and further records were made. Finally, atropin was injected, or the vagus nerves tied, in order to determine the effect of vagal release on the arrhythmias that were produced. After the completion of the experiment, the heart was removed and careful note made of the position of the woolen threads. In addition, the tissue lying beneath these electrodes was removed for histologic examination in order to determine whether they actually lay on the sino-auricular node. It may be stated at once that in none of the experiments was the pacemaker of the heart displaced from its normal site at the head of the sino-auricular node.

RESULTS

EXPERIMENT 1.—A dog weighing 30 pounds (13.6 kg.) was anesthetized with 90 mg. of morphin and 14 gm. of urethan. The upper electrode on the surface of the heart was 2 mm. below the cavo-appendicular junction, the lower one

10. Lewis, T.; Oppenheimer, B. S., and Oppenheimer, A.: The Site of Origin of the Mammalian Heart Beat; the Pacemaker in the Dog. Heart **2**:147, 1910-1911.

4 mm. below the upper, both lying on the sulcus terminalis. Histologic examination of the tissue lying beneath these threads proved that they were situated on the sino-auricular node. Figure 1 is a photograph of the heart after removal from the body, showing the position of the woolen threads.

Following the injection of 1 c.c. of pituitary extract, there appeared in a number of records an arrhythmia that was typical of so-called sino-auricular block. *In no instance was there any evidence of sinus activity during the long pauses, which were approximately twice the length of the normal cycles.* Figure 2 illustrates the arrhythmia that was seen, the upper curve being a record taken by Lead II, the lower one a record taken from the electrodes placed directly on the sino-auricular node. It is clearly seen that there is a complete absence of demonstrable activity in the sino-auricular node as well as in the auricle as a whole during the long pause. In Table 1 are the measurements of a number of successive cycles in several of the records that were taken in this experiment, the figures in heavy type being those of the long cycles.

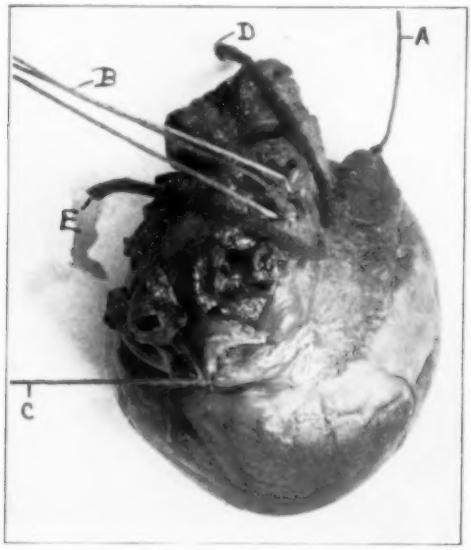

Fig. 1.—Heart of dog used in Experiment 1 (× three-fourths, approximately): *A*, *B* and *C*, strings attached to tip of right auricle, superior vena cava and inferior vena cava, respectively; *D* and *E*, upper and lower woolen threads attached to surface of heart, both lying on sulcus terminalis, just below cavo-appendicular junction; histologic examination of tissue lying beneath these threads showed that they were situated on sino-auricular node.

In Figure 3 are charted the measurements which are given in Table 1 (except those of Record 12).

This figure demonstrates more clearly perhaps the changes that occurred during the experiment. Record 3 is one taken before the administration of pituitary extract; it shows but slight variation in the length of the cycles. Records 5 and 6 are quite typical of sino-auricular block, the long pauses having about twice the duration of two average short cycles. Record 10, taken when the effect of the pituitary extract was wearing off, is that of an ordinary sinus arrhythmia. Record 11 was taken after the injection of a second cubic centimeter

of pituitary extract. The curve of the cycles differs from those of Records 5 and 6, in that following the long pause there is no rapid return to the normal rhythm; instead there is a more gradual slope in the descending limb of the curve. Finally, Record 13 shows the complete disappearance of the arrhythmia after vagal paralysis.

EXPERIMENT 2.—A dog weighing 34 pounds (15.4 kg.) was anesthetized with morphin, 48 mg., pantopon, 21 mg., and urethan, 15 gm. The upper electrode on the surface of the heart was 2 mm. below the cavo-appendicular junction, the lower one 5 mm. below the upper one, both lying on the sulcus terminalis. Histologic examination of the tissue beneath the electrodes proved that they were resting on the sino-auricular node.

TABLE 1.—*Measurements of Successive Cycles in Several Records Taken Before and After the Injection of Pituitary Extract in Experiment 1*

Record	Measurements of P-P Intervals * Seconds					Remarks
3	0.52	0.54	0.52	0.51	0.51	Before injection of pituitary extract
	0.51	0.50	0.47	0.46	0.51	
	0.46	0.50	0.49	0.49		
5	0.70	0.70	0.71	**1.41**	0.65	Records 5-10 taken after 1 c.c of pituitary extract
	0.73	0.70	**1.36**	0.71	0.67	
	0.83	0.62	**1.42**	0.68	0.70	
6	0.61	0.61	0.68	**1.57**	0.67	
	0.80	0.62	0.72	**1.43**	0.66	
	0.68	0.69	0.72	0.68	0.90	
	0.90	0.69	0.72	0.69	**1.41**	
	0.64	0.62	**0.57**			
10	0.59	0.53	0.66	0.62	0.73	15 minutes after 1 c.c. of pituitary extract was injected
	0.71	0.58	0.67			
11	0.61	0.60	0.61	**1.39**	0.96	Records 11 and 12 taken after a second cubic centimeter of pituitary extract was injected
	0.75	0.86	0.72	0.86	0.73	
	0.76	0.72	0.78	0.68		
12	0.57	0.62	0.60	0.66	**1.44**	
	0.78	0.89	0.60	1.00	0.75	
	0.73	0.74	0.84	0.72	0.86	
	0.67	0.66	0.64	0.61		
13	0.40	0.40	0.40	0.39	0.39	After the injection of 3 mg. of atropin intravenously
	0.39	0.39				

* The figures in this and the subsequent tables are to be read from left to right.

The normal records showed a moderate irregularity due to sinus arrhythmia. Following the injection of pituitary extract, there appeared in successive records an arrhythmia which is rather difficult to classify. The irregularity seemed to be due to a typical sino-auricular block, or to an unusual sinus arrhythmia, or perhaps to a combination of both types of irregularity. *In no record, out of the number that were taken, was there any evidence in the electrocardiographic record of activity of the sinus node during the long cycles.* In other words, the records showed, as did those of the experiment described above, that the dropping out of the auricular beat during the long pauses is not due to failure of conduction of the impulse from sinus to the auricle; it is due rather to failure of an impulse to arise in the sino-auricular node.

In Table 2 are given the measurements of successive cycles in several of the records taken during this experiment. These (except Record 12) are charted in Figure 4. Only the first of each group of long cycles is given in heavy type.

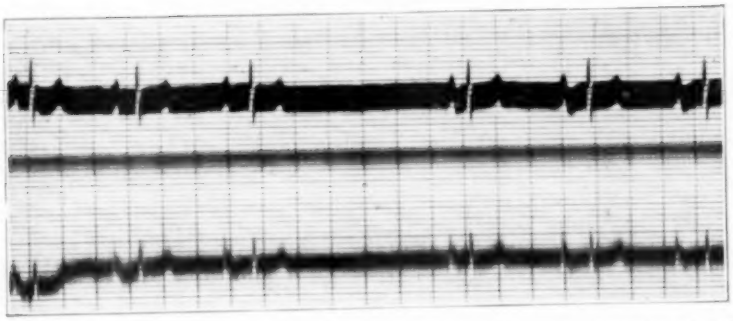

Fig. 2.—Upper curve, record from Lead II; lower, from electrodes placed directly over upper end of sino-auricular node; records were taken simultaneously on the same film; time lines are 0.2 second apart; upper curve is standardized so that 1 millivolt equals 5 mm., lower so that 1 millivolt equals 4 mm.; slight distortion of time lines where two curves merge, probably due to error of adjustment of prisms; vibrations in lower record due to induced currents caused by operation of nearby electric fan.

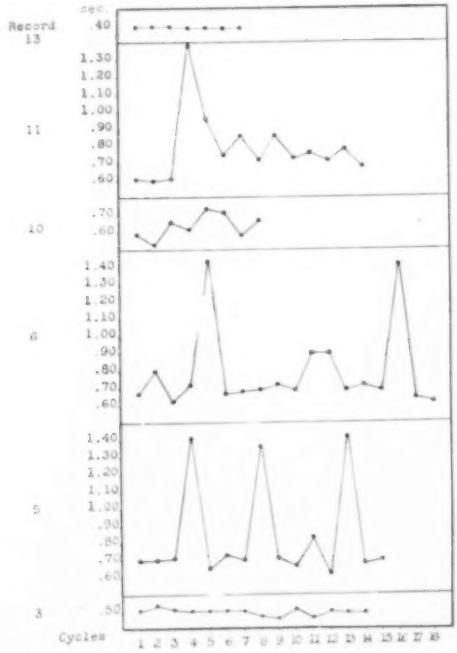

Fig. 3.—P-P intervals of successive cycles in records in Experiment 1; Record 3, before injection of pituitary extract; Records 5, 6, 10 and 11, after pituitary extract was given; Record 13, after vagus nerves were paralyzed by atropin (Table 1 and text).

The curves of the cycles in the records taken after injection of pituitary extract show many gradations between so-called sino-auricular block and sinus arrhythmia. In most of the curves, there is a steep upstroke, such as one sees in sino-auricular block; Records 3 and 5 are examples. But the onset of the long pause is not always so abrupt. Record 4 is an instance in which the third cycle, just before the long pause, is itself considerably longer than the two preceding cycles. In Record 13 the long cycle is ushered in by two moderately long cycles.

The downstroke of the curves is much more gradual, and it is difficult to judge when the long cycles, ordinarily attributed to sino-auricular block, leave off, and when the normal cycles begin. Record 11 of Figure 3 represents a type intermediate between those shown in Figure 4 and several in Figure 3. The downstroke of the curve is less steep than those of Records 5 and 6 of Figure 3; but it is more abrupt than those of Figure 4.

TABLE 2.—*Measurements of Successive Cycles in Several Records Taken Before and After the Injection of Pituitary Extract in Experiment 2*

Record	Measurements of P-P Intervals Seconds					Remarks
2	0.52	0.53	0.51	0.57	0.66	Before injection of pituitary extract
	0.74	0.71	0.69	0.63	0.63	
	0.61	0.61				
3	0.74	0.61	0.53	0.60	**1.05**	Records 3-13 taken after administration of 1 c.c. of pituitary extract
	1.03	0.96	0.94	0.90	0.86	
	0.70	0.54	0.55	0.75	**1.31**	
	1.29	1.04	1.00	0.96		
4	0.66	0.39	0.94	**1.34**	1.22	
	1.09	1.08	0.95	0.96	0.92	
	0.74	0.64	0.70	**1.20**	1.36	
	1.22					
5	0.72	0.68	**1.38**	1.48	1.40	
	1.30	1.17	1.20	1.16	0.99	
	0.70	0.77	0.80	0.78	0.86	
	1.05	1.27				
12	0.68	0.59	0.75	0.78	**1.00**	
	1.00	0.99	0.77	0.69	0.60	
	0.58					
13	0.61	0.58	0.80	0.74	**1.09**	
	1.04	0.96	0.84	0.73	0.62	
	0.58	0.71	0.70			
17	0.40	0.40	0.41	0.41	0.41	After administration of 1 mg. of atrophin
	0.41	0.41	0.41	0.41		

There were numerous dropped ventricular beats in addition to the foregoing irregularities following the administration of pituitary extract. Under the influence of atropin, the rhythm became entirely normal.

EXPERIMENT 3.—A dog weighing 22 pounds (10 kg.) was anesthetized with pantopon, 64 mg., and urethan, 10 gm. The upper electrode was placed 2 mm. below the cavo-appendicular junction, the lower one 6 mm. below the upper, both on the sulcus terminalis. Histologic examination of the tissue beneath the electrodes on the surface of the heart revealed sino-auricular nodal tissue.

This experiment may be described briefly. There was a slight irregularity of the rhythm which was accentuated after the administration of pituitary extract, and which disappeared when the left vagus nerve was tied. Table 3 contains the essential data which are illustrated in Figure 5.

Record 6, taken after the giving of pituitary extract, shows in the fourth cycle an abrupt rise, but the long cycle is scarcely long enough to be considered comparable to those of sino-auricular block; it is the record, then, of a definite but not unusually marked sinus arrhythmia. In this record, too, the auricular and sinus activities were synchronous.

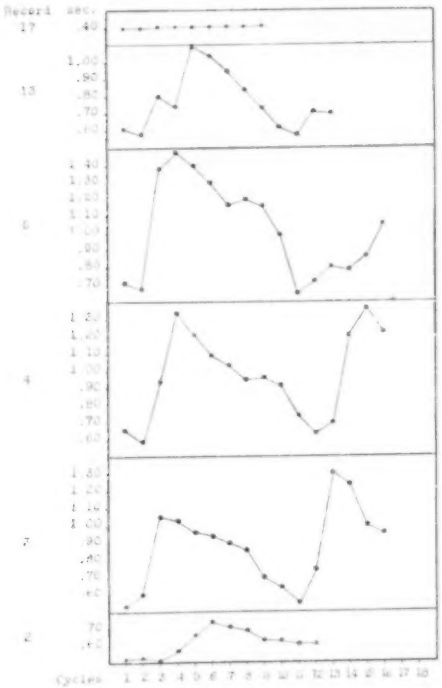

Fig. 4.—P-P intervals of successive cycles in records in Experiment 2: Record 2, before injection of pituitary extract; Records 3, 4, 5 and 13, after injection of pituitary extract; Record 17, after administration of atropin (Table 2 and text).

TABLE 3.—*Measurements of Successive Cycles in Three Records Taken During Experiment 3*

Record	Measurements of P-P Intervals Seconds					Remarks
3	0.35	0.37	0.46	0.46	0.45	Before injection of pituitary extract
	0.47	0.48	0.45	0.39	0.39	
	0.37					
6	0.53	0.52	0.56	0.82	0.62	After injection of 1 c.c. of pituitary extract
	0.56	0.67	0.53	0.53	0.55	
14	0.31	0.30	0.31	0.30	0.31	After the left vagus nerve was tied
	0.31	0.32	0.31	0.31		

COMMENT

It may be fairly stated that the arrhythmia of Experiment 1 was typical of the one to which the term sino-auricular block has been given; and the arrhythmia seen in Experiment 2 was closely related to so-called sino-auricular block, if not absolutely characteristic. It may also be accepted that these irregularities of rhythm, arising from vagal stimulation, were brought about in a manner essentially identical with that which underlies clinical sino-auricular block. If these assumptions are valid, then the theory of sino-auricular block, as it is applied to the clinical case, is thrown out of court. There is no evidence from the records that I have obtained that during the long cycles, impulses arising in the sinus node are impeded in their transmission to the auricle. The simultaneous records show, rather, that there is a complete cessation of activity of the sinus node, so far as such activity is demonstrable by the electrocardiograph. As I have indicated before, it is possible that clin-

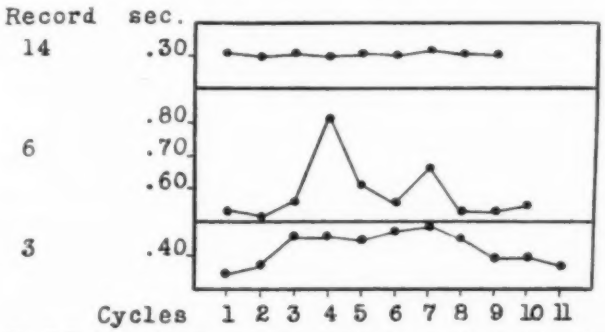

Fig. 5.—P-P intervals of successive cycles in records in Experiment 3; Record 3, before injection of pituitary extract; Record 6, after injection of pituitary extract; Record 14, after left vagus nerve was tied (Table 3 and text).

ical cases might occur in which there was actually a block between sinus and auricle. Most, if not all, of the reported cases almost certainly do not fall into this group, and for this reason the term sino-auricular block is an incorrect one. Sino-auricular standstill would be a more accurate expression of the clinical condition.

It has been observed before [2] that so-called sino-auricular block is frequently associated with sinus arrhythmia. The experiments I have reported point rather definitely to the reason for this coincidence. One can detect in a series of curves practically all transitions between typical sino-auricular block and typical sinus arrhythmia. So-called sino-auricular block is really a special form of sinus arrhythmia, and in these experiments, as in most clinical cases, both irregularities are due to the influence of the vagus nerves on impulse formation in the sino-auricular

node. While so-called sino-auricular block is usually due to vagal influence, it is not necessarily always brought about by this cause. Experimentally, I have observed on one occasion the appearance of this arrhythmia in the late stage of anoxemia, when the heart was completely under the influence of atropin;[11] but in this experiment, too, there was definite evidence that impulse formation in the sino-auricular node was being depressed by direct effect of anoxemia on the myocardium.

SUMMARY AND CONCLUSIONS

1. When so-called sino-auricular block comparable to that seen clinically is produced experimentally, there is no evidence that a block exists between the sino-auricular node and the auricles. Records taken simultaneously from Lead II, and from electrodes placed directly on the sino-auricular node, show a complete absence of demonstrable sinus activity during the long pauses. The arrhythmia is due, not to failure of conduction from sinus to auricles, but to failure of an impulse to arise in the sino-auricular node during the long pause.

2. Since "sino-auricular block" does not properly designate the arrhythmia to which it has been applied, in most, if not all, clinical cases it is a term that should be dropped. "Sino-auricular standstill" is suggested as a more accurate expression of the condition as it is seen clinically.

3. Practically complete transitions between sino-auricular standstill and sinus arrhythmia may be obtained in a series of records taken after the injection of pituitary extract. This is due to the fact that both irregularities occur as the result of vagal influence on impulse formation in the sino-auricular node. Sino-auricular standstill is merely a special form of sinus arrhythmia.

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11. Resnik, W. H.: Observations on the Effect of Anoxemia on the Heart, III, Changes in the Auricles, with Particular Reference to the Relationship Between Anoxemia and Auricular Fibrillation, to be published.

IDENTIFICATION OF THREE TYPES OF MONONUCLEAR PHAGOCYTES IN THE PERIPHERAL BLOOD*

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ST. LOUIS

The presence in the peripheral blood of a relatively large number of granular polymorphonuclear leukocytes and of lymphocytes has greatly facilitated the investigations by means of which these white blood cells have been traced to the bone marrow and to the lymphoid tissue, respectively. The determination of the origin of the mononuclear phagocytes which are much less numerous in the normal blood has been attended with greater difficulty. Ehrlich,[1] by the use of differential staining and especially by the method employing the triacid stain, recognized in the blood a large mononuclear leukocyte which he thought came from the spleen and the bone marrow and which was in the circulating blood transformed into the polymorphonuclear leukocyte. He observed further that some mononuclear leukocytes had indentations in the nuclei together with a few fine granules in the cytoplasm, and these he regarded as the transitional forms between the mononuclear leukocyte and the polymorphonuclear neutrophil. The two terms "large mononuclear leukocyte" and "transitional leukocyte" have been retained rather persistently by clinical hematologists although the view held by Ehrlich of their origin has generally been considered incorrect.

Mallory[2] in 1898 named the mononuclear phagocytes of typhoid lesions "endothelial leukocytes." Since that time cumulative evidence has given much support to the view that the mononuclear phagocytes in the tissues and blood arise from endothelium. Aschoff and Kiyono[3] by vital staining with colloidal dyes traced these free cells to the reticuloendothelium. Sabin,[4] in the chick embryo, saw endothelial cells detach themselves from the walls of veins and become free in the lumina of the vessels. After marking the vascular endothelium by the intravenous

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1. Ehrlich, P.: Ueber die Specifischer Granulationen des Blutes, Arch. f. Anat. u Physiol., 1879, p. 571.

2. Mallory, F. B.: Histological Study of Typhoid Fever, J. Exper. Med. **3**:611, 1898.

3. Aschoff, L., and Kiyono, K.: Zur Frage der Grossen Nononukleären, 1913, Folia. Haematol. **15**:383, 1913.

4. Sabin, F. R.: Studies on the Origin of Blood Vessels and of Red Blood Corpuscles as Seen in the Living Blastoderm of Chicks During the Second Day of Incubation, Contribution to Embryology **9**, Carnegie Inst. Publication 272, p. 213.

injection of carbon in suspension, I [5] observed the carbon laden cells to undergo mitosis and to separate and assume the same appearance as the free phagocytes.

Recently Sabin, Doan and Cunningham [6] gave the first noteworthy evidence that these phagocytes are not of a single type by demonstrating in peritoneal exudates with supravital stains a cell with the neutral red granules grouped in the *hof* of the nucleus in the form of a rosette in distinction to a second type with neutral red granules scattered diffusely. The final conclusions reached by Cunningham, Sabin and Doan [7] are that the rosette type of cell is the monocyte and that it arises in common with the granular leukocytes and lymphocytes from a mesenchymal or reticular stem cell and that the clasmatocytes and the erythroblastic cells arise from endothelium. They were successful in demonstrating the rosette cell in the spleen, bone marrow, omentum and liver but did not identify it in the lymph nodes.[8] I [9] repeated their experiments on the peritoneal exudates of rabbits and in addition to the two types described by them found a third "hyaline" type with little affinity for neutral red. Examination by me of the lymph nodes, spleen and liver, fresh and in paraffin sections that had the dye mordanted in them, gave evidence which appears to be conclusive that the rosette type of cell first described by Sabin and her co-workers, Doan and Cunningham, arises from the reticulo-endothelium of the lymph nodes. The free cells of this type were seen to undergo mitosis but only in the lymph node have they been demonstrated to arise from fixed tissue.

In rabbits that have received many intraperitoneal injections of whole rabbit's blood evidence of the origin of the rosette cell from the reticulum of the lymph nodes seems beyond controversy. In the enlarged nodes of such rabbits perfect rosettes are present in the large attached endothelial cells. The large rosette cells that are present both in peritoneal exudates and in lymph nodes are essentially abnormal, stimulated forms but such rosettes afford direct evidence of the identity of the two cells. It was observed in rabbits previously injected intravenously with large amounts of India ink that one-half the hyaline

5. McJunkin, F. A.: The Origin of the Phagocytic Mononuclear Cells of the Peripheral Blood, Am. J. Anat. **25**:27 (Jan.) 1919.

6. Sabin, F. R.; Doan, C. A., and Cunningham, R. S.: The Separation of the Phagocytic Cells of the Peritoneal Exudate into Two Distinct Types, Proc. Exper. Biol. & Med. **31**:330, 1924.

7. Cunningham, R. S.; Sabin, F. R., and Doan, C. A.: The Development of Leukocytes, Lymphocytes and Monocytes from a Specific Stem Cell in Adult Tissues, Contribution to Embryology, Carnegie Inst. of Wash. Publication 361, p. 227.

8. Cunningham, Sabin and Doan (Footnote 7, p. 259).

9. McJunkin, F. A.: The Origin of the Mononuclear Phagocytes of Peritoneal Exudates, Am. J. Path. **1**:305 (May) 1925.

cells may contain ink particles at a time when less than one in a hundred other mononuclear phagocytes carried ink granules. The Kupffer cells of the liver react to neutral red, to trypan blue and to carbon in suspension after the fashion of the hyaline type. These observations indicate that the "hyaline" cells present in the exudate are derived from vascular endothelium of the type that lines the sinusoids of the liver. The "hyaline" cell, especially when phagocytic, may acquire more of the neutral red, but the phagocyte, abundant in the exudates and characterized by a diffuse neutral red granulation, was thought to arise chiefly through a dispersion of the neutral red granules of the rosette cell with loss of the wreath appearance. At an earlier date I [5] adopted the Mallory term of "endothelial leukocyte" for the mononuclear phagocytes. It is the only term that names accurately the origin of these phagocytes. Separation of the group into the lymphendotheliocytes of lymph vascular origin and the hemendotheliocytes of blood vascular origin now appears possible, at least under certain experimental conditions.

Sabin, Cunningham and Doan constantly refer to the rosette type of cell as the monocyte of the rabbit's blood and apply to it this term, which Naegeli [10] applied to the granular oxydase reacting mononuclear cell of peripheral human blood. Simpson [11] found no mononuclear cells present in the blood of the rabbit which give the oxydase reaction and I [9] found this to be true for the peritoneal exudate cells so far as the peroxydase reaction with benzidin is concerned. By comparison of the mononuclear cells of human blood that react to benzidin with those that ingest carbon in vitro the conclusion was reached by myself and Charlton [12] in 1918 that the benzidin reacting cell of the peripheral blood was the endothelial phagocyte of the tissue. Subsequently in 1924 I [13] devised a method by which peroxydase staining was made applicable to paraffin sections of human tissue and found that only small foci of reacting cytoplasm were demonstrable in occasional Kupffer cells and that no cells of the human lymph gland reacted. The irregular reaction seen occasionally in phagocytic endothelium had the appearance of phagocytized material. In the paraffin sections reacting mononuclear phagocytes were seen only in the spleen. This discrepancy between the blood and the tissues as regards peroxydase staining with benzidin was not explained at that time. The present investigation of the blood was therefore undertaken to determine first the occurrence in the blood of

10. Naegeli, O.: Blutkrankheiten und Blutdiagnostic, Berlin, 1923.

11. Simpson, M. E.: The Experimental Production of Macrophages in the Circulating Blood, J. M. Res. **43**:77 (April-May) 1922.

12. McJunkin, F. A., and Charlton, A. G.: The Practical Identification of Endothelial Leukocytes in Differential Blood Counting, Arch. Int. Med. **22**:157 (Aug.) 1918.

13. McJunkin, F. A.: Peroxydase Staining with Benzidin in Paraffin Sections of Human Tissue, Anat. Rec. **24**:67, 1922.

hemendotheliocytes and the lymphendotheliocytes, which had previously been demonstrated in peritoneal exudates,[14] and, second, the relationship of these two phagocytes to the mononuclear benzidin reacting phagocytes of human blood.

METHODS

Method A. Phagocytosis in Vitro.[14]—To 2 c.c. of 3.8 per cent. sodium citrate solution in a graduated centrifuge tube, 3 c.c. of blood is added. The citrated blood is mixed with 1 drop of India ink (Higgins') and the mixture incubated at 37 C. for ten minutes. The mixture is centrifugated at low and then at high speed and the tube, after the careful complete removal of the supernatant liquid, returned to the incubator for fifteen minutes. The leukocytic layer is removed with a capillary pipet and smears are made on slides. It is essential to spread out completely the droplet and to guard against the smear reaching the edge of the slide; otherwise, most of the leukocytes may be lost. A small bulb pipet with the capillary at an angle of 135 degrees is convenient for removal of the liquid from the centrifuge tube. The 3 c.c. of blood may be obtained from the finger of some person by "milking" after puncturing deeply near the thumb angle of the nail. In the guinea-pig the blood is obtained from the heart. By placing a rabbit head downward held firmly in a suitable box this amount of blood flows readily from the marginal ear vein after insertion and removal of a large needle. The phagocytosis obtained with human blood is quite complete but is very much less satisfactory in the case of the guinea-pig and the rabbit.

Method B. Peroxydase Staining of Smears with Benzidin.[15]—The fresh preparation of the leukocytic layer is covered for from thirty to sixty seconds with about 10 drops of an alcoholic solution of benzidin consisting of 100 mg. of dry benzidin dissolved in 25 c.c. of acetone-free 80 per cent. methyl alcohol that contains 1 drop of hydrogen peroxid. To the alcoholic solution 10 drops of distilled water is added and two minutes are allowed for the reaction to take place. In the case of guinea-pig or rabbit blood the reaction requires from five to ten minutes. Smears of guinea-pig or rabbit blood must be treated as soon as they become dry but the leukocytes of human blood react after several hours. The solution is washed off and hematoxylin (Harris' without acetic acid) applied for from twenty to sixty seconds. The hematoxylin is followed by 0.01 per cent. eosin solution for twenty seconds. The

14. McJunkin, F. A.: A Simple Technique for the Demonstration of a Phagocytic Mononuclear Cell in Peripheral Blood, Arch. Int. Med. **21**:59 (Jan.) 1918.

15. McJunkin, F. A.: A Benzidin-Polychrome Stain for Blood, J. A. M. A. **74**:17 (Jan. 3) 1920.

preparation may also be stained by allowing Wright's stain properly diluted to act for from five to ten minutes.

Method C. Peroxydase Staining with Benzidin in Paraffin Sections.[13]—The tissue, fixed for one day in 4 per cent. formaldehyd solution, is cut into small bits not more than 2 mm. in thickness, placed in 70 per cent. acetone solution for two hours, pure acetone for thirty minutes, benzene for twenty minutes, and 52 C. paraffin for twenty minutes. Thin sections are attached to slides by allowing them to dry over night at room temperature. The paraffin is removed with benzene (ten seconds) and acetone (ten seconds). The sections are covered with the diluted benzidin solution for five minutes, washed and stained with hematoxylin and eosin, as in Method B. After the eosin is washed off the excess of water is carefully removed with a soft cloth and the preparation dehydrated with acetone (ten seconds) and benzene (ten seconds), when it is mounted in balsam. To dehydrate in so short a time the solutions are run over the preparations from dropping bottles.

Method D. Supravital Staining of Leukocytes with Neutral Red.—In a centrifuge tube, to 10 c.c. of a neutral red solution consisting of 19 parts of saline solution and 1 part of saline solution saturated with neutral red (Gruebler) 2 or 3 drops of the fresh leukocytic layer without ink is added. After being mixed and allowed to stand for about ten minutes the cells are sedimented in the centrifuge tube and all of the supernatant liquid except about 2 drops is removed. With a platinum loop the cells are mixed with the liquid and a small droplet is transferred to the center of a cover glass rimmed with petrolatum and at once inverted on a slide for examination with oil immersion lens. The cells are permitted to remain in the citrate the shortest time possible.

Method E. Zenker-Formaldehyd Solution (Formol) Method of Mordanting the Supravital Dye in Smears.[9]—The reaction has usually reached the maximum intensity after about forty-five minutes contact with the dye. Heavy smears are made and just as drying becomes complete they are dropped into a solution consisting of 5 parts of 40 per cent. formaldehyd and 95 parts of Zenker's fluid without acetic acid. After two hours' fixation the preparations are washed and at once covered with hematoxylin (Harris' without acetic acid) for two minutes. The hematoxylin is washed off and the slides immediately blotted dry. The dye granules are well preserved but the nuclear staining is faint.

Method F. Iodin Vapor Method of Mordanting the Supravital Dye in Smears.—Tissue cultures stained supravitally have been successfully fixed in iodin vapor for microscopic examination and photographic pur-

poses by Lewis.[16] The smears of the fresh leukocytic layer, as they become dry, are placed for thirty minutes above iodin crystals in a glass jar 4 inches (10.1 cm.) in diameter and 6 inches (15.2 cm.) high with a ground glass cover. They are removed from the iodin vapor, covered with hematoxylin (Harris' without acetic acid) for one minute, washed and at once blotted dry with filter paper. The nuclei are well stained with hematoxylin, but the dye granules are apt to range from brown to black. Lewis does not mention using a nuclear stain.

Method G. Zenker-Formaldehyd Solution (Formol) Method of Preserving Supravital Stains in Paraffin Sections.[9]—Under anesthesia or immediately after removal from the living body a saline solution saturated with neutral red (Gruebler) is injected into the lymph nodes, spleen or liver until the tissue becomes distended by the liquid. A second injection after a few minutes is advisable. After a half-hour the tissue is placed for twelve hours in Zenker-formaldehyd solution (formol) consisting of 15 c.c. of 40 per cent. formaldehyd and 85 c.c. of Zenker's fluid without acetic acid. It is then cut into pieces not to exceed 3 mm. in thickness and transferred to Zenker's fluid without acetic acid for from twelve to twenty-four hours. The bits of tissue are then placed in pure absolute acetone for one hour (two changes), in benzene for twenty minutes and in 52 C. paraffin for twenty minutes. Extra thin sections are then attached to slides with albumin fixative by allowing to dry over night at room temperature. To stain, the paraffin is removed with xylene (ten seconds) and pure acetone (ten seconds). After immersion in water (five seconds) the slide is stained very lightly with hematoxylin (Harris' without acetic acid) for about five seconds. The section is then dehydrated with pure acetone for ten seconds, at once covered with xylene for ten seconds and mounted in balsam. To limit the action of the acetone and xylene to these times, the slides are stained singly and the solutions run over them from dropping bottles.

MONONUCLEAR PHAGOCYTES OF RABBIT'S BLOOD

A normal rabbit, Rabbit H 16 (accompanying table), was one of several full grown rabbits examined. In supravital films (Method D) before any injections had been made, there were present 3 per cent. lymphendotheliocytes (the count from a fresh preparation). The blood of this rabbit was frequently examined to determine the occurrence in it of a carbon marked mononuclear cell that did not react to neutral red (Method A, followed by Method D), but no such cell was found. On this basis it was concluded that hemendotheliocytes were not present.

16. Lewis, W. H.: Endothelium in Tissue Cultures, Am. J. Anat. **30**:39. 1922.

Satisfactory phagocytosis of carbon has rarely been obtained in the citrated blood of the rabbit or guinea-pig. Examination of smears (Method B) showed an absence of benzidin reacting mononuclear cells. All mononuclear phagocytes reacting to neutral red were considered to be of the lymphendothelial type. In the fresh films they are about the size of the polymorphonuclear leukocytes with most of the dye in a focus but frequently dye granules are present elsewhere in the cytoplasm. In an occasional cell the dye is in the form of a characteristic

Rabbit H 16; Weight, 2,010 Gm.

Date	Treatment	Examination of Blood
4/ 6/25	10 c.c. of blood intraperitoneally (after blood examination)	Lymphendotheliocytes,* 3 per cent; hemendotheliocytes,† none
4/ 7/25	10 c.c. of blood intraperitoneally...	
4/ 8/25	40 c.c. of blood intraperitoneally...	
4/10/25	4 c.c. of ink intravenously ‡ (after blood examination)	Lymphendotheliocytes, 7.2 per cent.; hemendotheliocytes, none
4/13/25	4 c.c. of ink intravenously (after blood examination)	
4/14/25	.....	Lymphendotheliocytes, 4 per cent.; hemendotheliocytes, 1 in 15 minutes
4/20/25	4 c.c. of ink intravenously.....	
4/21/25	.....	Lymphendotheliocytes, 4.2 per cent.; hemendotheliocytes, 3 in 15 minutes
4/22/25	.....	Unchanged
4/27/25	4 c.c. of ink intravenously.....	
4/28/25	4 c.c. of ink (after blood examination)	Lymphendotheliocytes, 3 per cent.; hemendotheliocytes, 1 per cent.
4/29/25	15 c.c. of blood intraperitoneally...	Detailed count of 1,000 cells: granular leukocytes, 59.1 per cent.; lymphocytes, 27.9 per cent.; lymphendotheliocytes, 3.6 per cent.; hemendotheliocytes with carbon, 3.7 per cent., and hemendotheliocytes (?) without carbon, 5.7 per cent.§
4/30/25	20 c.c. of blood intraperitoneally... 5 c.c. of ink intravenously.....	
5/ 1/25	.....	Lymphendotheliocytes, 7 per cent.; hemendotheliocytes, 4 per cent., with much carbon
5/ 2/25	20 c.c. of blood intraperitoneally...	
5/ 4/25	20 c.c. of blood intraperitoneally... 5 c.c. of ink intravenously.....	
5/ 5/25	.....	Lymphocytes, 20.5 per cent.; granular leukocytes, 67.6 per cent.; lymphendotheliocytes, 6.8 per cent.; hemendotheliocytes, with carbon, 2.2 per cent.; hemendotheliocytes (?), without carbon, 2.9 per cent.
5/ 6/25	Chloroform.....	Groin lymph nodes showed a slight tinge of gray and measured 3 by 5 by 9 mm.

* Neutral red may or may not be arranged in form of rosette, Method D.
† Methods A and D.
‡ The ear vein was used.
§ Without ingested carbon identification of the hemendotheliocytes is uncertain.

rosette. These wreath forms are uncommon in normal human or guinea-pig blood. The wreath form of this cell is apt to be larger than the granular leukocyte while the cell with a dye focus lacking the rosette or wreath appearance is usually slightly smaller. The interpretation of this observation is that the smaller leukocyte is the older one. The lymphocytes may contain no neutral red or they may show from one to several small granules in a focus of cytoplasm. In the lymphendotheliocytes as seen in fixed smears the dye appears as large, heavy dye masses.

Following three daily injections, April 6, 7 and 8, of whole rabbit's blood into the peritoneal cavity of Rabbit H 16, the lymphendotheliocytes numbered 7.2 per cent., April 10 (table). No hemendotheliocytes were present. April 14, the lymphendotheliocytes had dropped to 4 per cent. April 10, 13, 20, 27, 28 and 30, the rabbit received injections of 4 or 5 c.c. of India ink (Higgins') into the ear vein. April 29, the hemendotheliocytes marked by carbon numbered 3.7 per cent. When this number is present there is an impression that these cells contain more carbon than the lymphendotheliocytes, and, since the cytoplasm is devoid of neutral red, the cell is entirely distinctive in supravital preparations. Careful observation is often necessary to outline the hyaline cytoplasm. In a smaller, 1,250 gm. rabbit that received only two injections of 5 c.c. of ink, these cells numbered 5.2 per cent. eighteen hours after the second dose. The usual experience has been that the appearance of these cells in the peripheral blood is quite irregular and follows large doses of the suspensions. April 29, there were present 5.7 per cent. carbon-free mononuclear cells that resembled those with carbon and on incubation with dilute ink some of these appeared to ingest the carbon particles, but the results obtained by this method were not conclusive. The only certain method of identifying these cells in the rabbit is by the intravenous injection of carbon. These hyaline carbon marked hemendotheliocytes vary in size (Fig. 1, *A* and *B*). A great majority of those appearing in the peripheral blood have a spherical nucleus and often a relatively abundant cytoplasm.

Examination, March 6, of cells in fixed supravital smears from a normal rabbit, H 16, by Method F revealed lymphocytes, 32.1 per cent.; polymorphonuclears, 63.1 per cent., and lymphendotheliocytes, 4.8 per cent. In fresh supravital stains the percentage of lymphendotheliocytes was 3. It has been the usual experience that a larger number of neutral red reacting mononuclear cells can be identified in the fixed preparations. They vary from a size intermediate between lymphocyte and polymorphonuclear leukocyte to a size somewhat larger than the polymorphonuclear. The amount of dye is large (Fig. 1, *C* and *D*) and is placed unilateral with a flattened side of the nucleus toward it; or the dye granules may lie in a V shaped depression of the nucleus. In the lymphocytes from one to several minute dye granules appear at one point in the cytoplasm near the nucleus (Fig 1, *C*). The larger of the lymphocytes may reach the size of the small lymphendotheliocytes. In the cells that present a neutral red rosette in the fresh specimen there is a central vacuole in a fixed preparation (Fig. 1, *C*). The neutral red granules in the polymorphonuclear leukocytes are small but distinct (Fig 1, *D*). It is a noteworthy fact that there is an occasional polynucleated cell that contains much neutral red and that in smears treated with benzidin there is an occasional

nongranular polymorphonuclear leukocyte that does not react. These two findings are viewed as an indication that the nuclei of some of the lymphendotheliocytes may become broken into lobes and assume a polynucleated form. A great many smears both of whole blood and of the leukocytic layers have been treated with benzidin (Method B). An examination of these shows that benzidin reacting mononuclear cells are not present in the blood of the normal rabbit.

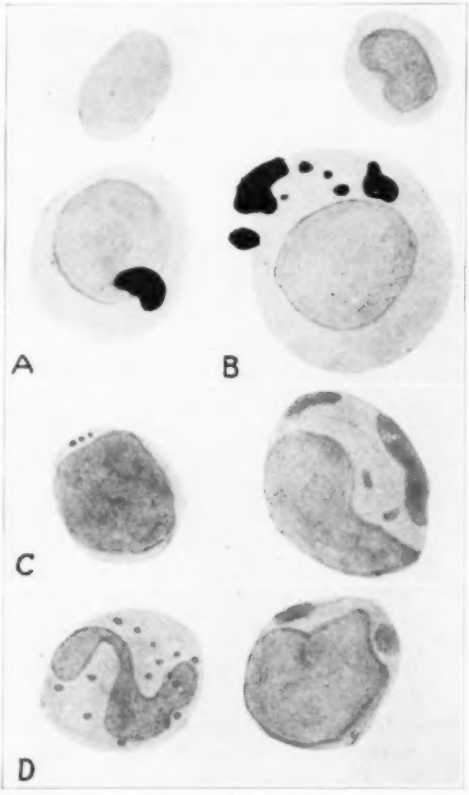

Fig. 1.—*A* and *B*, smear of peripheral blood of Rabbit H 16 (table) made April 29, 1925; *A*, hemendotheliocyte with one large ink particle in homogenous (hyaline) cytoplasm; red blood corpuscles in same field; *B*, hemendotheliocyte of larger size containing carbon, with a lymphocyte in same field; *C* and *D*, smears of blood of normal rabbit supravitally stained (Method F); these are both considered to be lymphendotheliocytes since benzidin-reacting cells are not present in the blood of the rabbit; the dye masses are heavy when compared with the dye granules in the lymphocyte and in the neutrophil; Wright's stain; camera lucida. The size of the cells varies in different preparations and in different fields in the same smear; therefore, adjoining cells have been drawn for comparison.

The enlarged lymph nodes from the groin of Rabbit H 16 were examined by the supravital Method G. A great majority of the lymph-endothelial cells (attached) contain no carbon. They are large and present the usual dye focus (Fig. 3, *A*), which often in the fresh preparation appears as a distinct wreath. In an occasional large cell there is a wreath appearance in the paraffin section. In the areas to which the dye has penetrated in a dilute form there is no nuclear staining and the lymphocytes show no dye whatever although the foci in the endothelial cells are very distinct.

MONONUCLEAR PHAGOCYTES OF GUINEA-PIG'S BLOOD

In the blood removed from the right ventricle (thorax opened) of a normal guinea-pig, H 14, there were 4.3 per cent. neutral red nonlymphocytic mononuclear cells when examined fresh by Method D. Typical rosettes were not present and there was a greater tendency than in the rabbit for dye granules to appear at several points in the cytoplasm. In the normal guinea-pig and in human blood the dye granules have not been seen to arrange themselves in the rosette or wreath form of Sabin, Doan and Cunningham, although it is usual to find an aggregation of them at one point in the cytoplasm. By Method F in preparations fixed in iodin vapor the relative number of leukocytes is: lymphocytes, 77.3 per cent.; polymorphonuclears, 20.3 per cent., and nonlymphocytic neutral red mononuclears, 2.4 per cent. In this instance the discrepancy between the results of examination of fresh and fixed preparations is due to a failure to enumerate many of the lymphocytes in the fresh film. The cells with Kurloff bodies in both the fresh and the fixed preparations are characteristic.

The mononuclear phagocytes of the blood of the normal rabbit do not react with benzidin (Method B). In the peripheral blood of the guinea-pig occasional mononuclear cells that do react to benzidin were previously found by myself and Charlton.[17] The smears should be treated immediately with the benzidin and the diluted solution allowed to react for from five to ten minutes. In the leukocytic layer of a normal guinea-pig, H 14, 0.57 per cent. of the leukocytes were found to be benzidin reacting mononuclear cells. Those found were larger in size than the polymorphonuclear leukocytes. Since there were no large mononuclear cells that failed to react to neutral red those positive to the benzidin probably reacted also to the neural red. In the normal guinea-pig therefore almost all of the nonlymphocytic mononuclear cells are lymphendotheliocytes. Under certain pathologic conditions the relative number

17. McJunkin, F. A., and Charlton, A. G.: An Experimental Endothelial Leukocytosis in Guinea-Pigs, Arch. Int. Med. **24**:295 (Sept.) 1919.

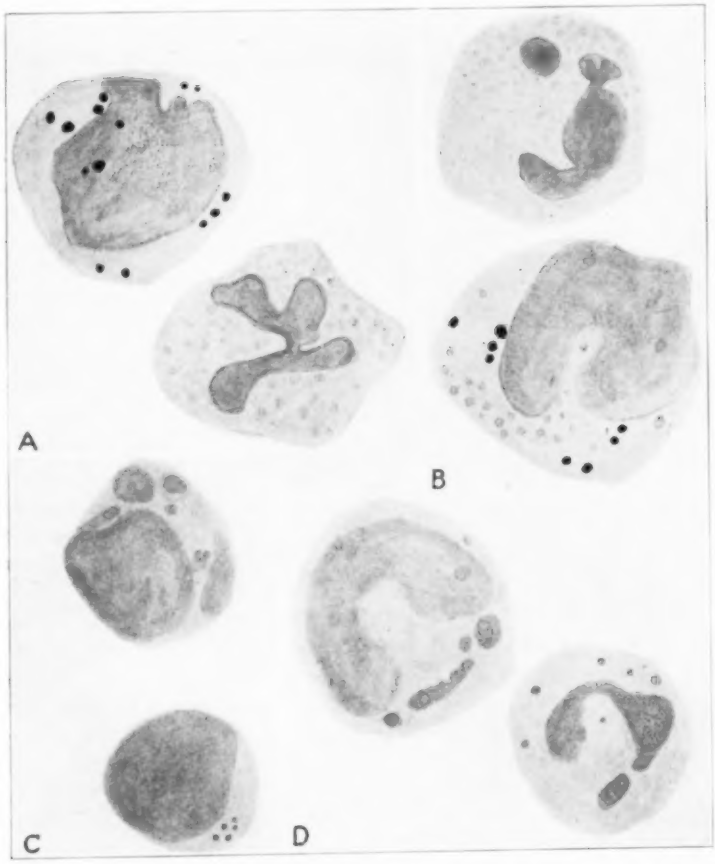

Fig. 2.—Human blood, from H 22; *A* and *B*, from preparation made by peroxydase staining after phagocytosis in vitro (Method A followed by Method B); *A*, lymphendotheliocyte and neutrophil, the former devoid of benzidin granules; *B*, benzidin-positive phagocyte, the monocyte of normal human blood, with neutrophil above; *C* and *D*, from a preparation made by supravital staining and subsequent fixation in iodin vapor (Method F); *C*, a lymphocyte with a few fine neutral red granules and a neutral red mononuclear phagocyte, probably a lymphendotheliocyte, with heavy dye masses. Such cells in fresh supravital films have no definite rosette but the dye is massed at one place in the cytoplasm. *D*, a polymorphonuclear with small dye granules and a mononuclear, probably a monocyte, with heavy dye granules. Since both lymphendotheliocytes and monocytes react to neutral red, the identification of the two in preparations treated with neutral red alone is not absolute.

of benzidin reacting mononuclears may be much increased. In an earlier report McJunkin and Charlton [17] found as many as 10 per cent. in the blood of guinea-pigs in the terminal stages of a general tuberculosis.

In a fresh preparation from a tuberculous guinea-pig, H 13, 8.3 per cent. of nonlymphocytic neutral red cells were found in the leukocytic layer prepared from the blood of the right ventricle (Method D). Many of these cells were larger than the polymorphonuclears and often there was a distinct rosette of dye granules made conspicuous by the abundant cytoplasm about the focus of dye. In other large cells the dye granules were much less localized. In direct smears of the whole blood made by snipping the ear there were 0.9 per cent. benzidin reacting mononuclear cells. Necropsy of this animal, infected with a human type tubercle bacillus (H 37) of rather low virulence, showed an extensive caseous tuberculosis of the groin lymph nodes with a few small tubercles in the spleen and liver. It has been a common experience in the examination of a number of guinea-pigs moderately tuberculous to find the lymphendotheliocytes considerably increased but with only a slight increase in the benzidin reacting mononuclears. It is only in tuberculous guinea-pigs a few days before death that a distinct increase in the benzidin type of cell takes place. For the demonstration of these cells in the guinea-pig it is much better to make the smears directly from the ear and to treat them at once with benzidin.

In the large mononuclear cells of the epithelioid type present in the tubercles of the spleen and in the tuberculous lymph nodes (Guinea-pig H 13), the neutral red granules viewed in paraffin sections with the supravital dye mordanted in them (Method G) are found to be arranged in beautiful rosette forms (Fig. 3, *B*). The dye usually penetrates only the peripheral portions of the epithelioid tubercle, where all the typical large epithelioid cells appear to be of the rosette type. In the spleen of this animal there is little caseation and the reaction consists almost entirely of these cells. In the liver the tubercles that are smaller are penetrated by cells that appear to be continuous with the vascular endothelium, and in their cytoplasm may be found a few neutral red granules scattered about much as they are in the Kupffer type of endothelial cell. There is direct evidence that both hemendotheliocytes and lymphendotheliocytes take part in tubercle formation in the guinea-pig, but it appears that it is the latter cell that enlarges to become the usual epithelioid cell in the epithelioid type of tubercle in the spleen and lymph node. Lewis [18] observed in incubated blood the development of epithelioid and giant cells from leukocytes present in the normal peripheral blood, including human. It was not shown which type undergoes these transformations.

18. Lewis, M. R.: The Formation of Macrophages, Epithelioid and Giant Cells from Leukocytes of Incubated Blood, Am. J. Path. **1**:91 (Jan.) 1925.

MONONUCLEAR PHAGOCYTES OF HUMAN BLOOD

It is only in human blood that the benzidin reacting leukocyte is the predominating mononuclear phagocyte. Charlton and I [12] compared the peripheral blood by the phagocytic method (Method A) with the same blood treated with benzidin (Method B) and found that the mononuclear cells with ingested carbon and those reacting to benzidin appeared to correspond in number and in general morphology. At this time the

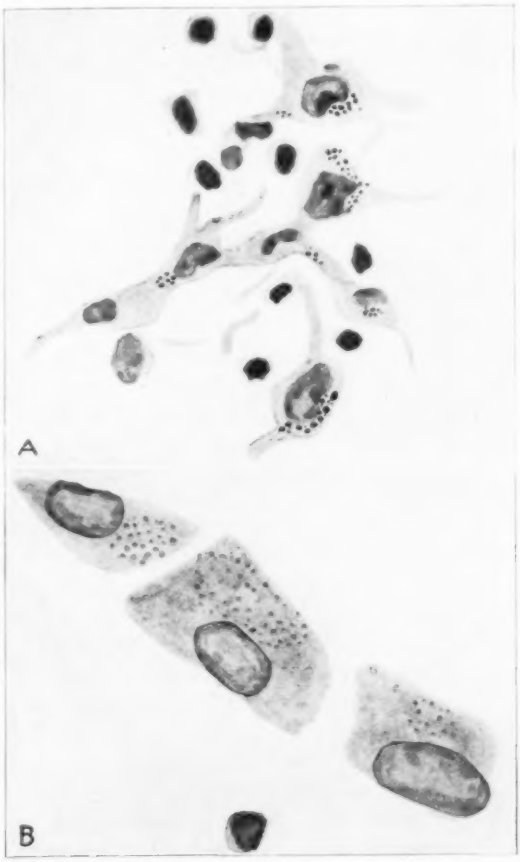

Fig. 3.—Sections of tissue with supravital dye mordanted in them (Method G): *A*, lymph node removed from groin of Rabbit H 16; the lymphocytes contain no dye but in the cytoplasm of the sinus endothelium there are foci of dye granules; *B*, spleen of tuberculous guinea-pig, H 13; cells at periphery of tubercle made up of epithelioid cells; spherical areas of fine neutral red granules in these large cells highly characteristic; lymphocyte below.

conclusion was reached that this benzidin reacting cell was identical with the carbon marked phagocyte that separates from the vascular endothelium. Subsequently, I [13] found in paraffin sections that neither the lymphatic endothelium nor the vascular endothelium reacted to benzidin. Later when I [9] determined that the neutral red reacting cells of peritoneal exudates of the rabbit did not color with benzidin it was thought not unlikely that the benzidin reacting cell was a different type from the endothelial leukocytes.

Examination of smears of human lymph nodes removed one hour after death by Method B showed only an occasional benzidin reacting mononuclear cell. In human lymph nodes removed at surgical operation and fixed immediately for examination by Method C, the reticulo-endothelium did not react to benzidin. The occasional mononuclear cell found in the heavy lymph node smears (about 1 per oil immersion field) which reacts to benzidin corresponds morphologically to the benzidin-positive ones of the blood and presents prominent brown granules scattered through the cytoplasm. The other numerous phagocytic cells present do not color with the benzidin. In an examination of lymph glands from a great many individuals by Method C and by staining with hematoxylin only, it has been observed that the endothelial cells lining the medullary sinuses frequently contain small ovoid or spherical bodies, which are apt to appear greenish or brownish. These bodies are sometimes surrounded by a delicate halo that suggests ingested material. In some instances they stain like bacteria while in other nodes they resemble pigment, but a positive reaction for iron has not been obtained. The nature of these structures which have the position in the cells of centrospheres is uncertain.

HUMAN BLOOD IN H 22

Many examinations were made on the normal adult male, H 22. The blood was usually obtained by gently "milking" the finger after deep puncture with a lance. A long series of examinations were first made by subjecting the carbon laden leukocytic layer to vital staining (Method A followed by D) in order to determine the occurrence in the normal peripheral blood of hemendotheliocytes. These examinations ended in the conclusion that this phagocyte with hyaline cytoplasm, which has little affinity for neutral red, is not present in normal human blood. It was stated in a preceding paragraph that the hemendotheliocyte is probably absent from the blood of the rabbit, but in the rabbit the evidence is less conclusive, owing to the partial failure of phagocytosis by Method A.

There is very active phagocytosis of carbon (Method A) by the cells of human blood. In a careful examination of smears of the carbon exposed leukocytes by the peroxydase method with benzidin (Method

B), it soon became evident that mononuclear cells phagocytic for carbon which did not react with the benzidin were present although a majority of the ink marked mononuclear cells did color with the benzidin. In an enumeration of 1,000 cells the following percentages were obtained: lymphocytes (no benzidin), 24.3; neutrophils and eosinophils (heavy benzidin with no carbon in eosinophils and little carbon in neutrophils), 70.8; mononuclear phagocytes (heavy benzidin and much carbon), 3.4; mononuclear phagocytes (no benzidin and much carbon), 1.1, and polymorphonuclear cells with no benzidin (unclassified), 0.4. Taken as groups the benzidin mononuclear phagocytes appear to differ somewhat from the nonbenzidin ones. The benzidin-positive cells average somewhat larger in size and their nuclear contour is often broken by deep indentations (Fig. 2, *B*), while the nucleus of the benzidin-negative cell may present only a flattened surface toward a wider expanse of cytoplasm, or the nuclear outline may be broken by one or more shallow V shaped depressions (Fig. 2, *A*). In the incubated specimen, in which ameboid motion is likely to distort both nuclear and cytoplasmic outlines, there appears to be no feature other than the reaction to benzidin which will serve to distinguish the two types of cells. It also is certain that some of the nonbenzidin phagocytes can be differentiated from lymphocytes only by the ingested carbon. Where the carbon particles are very numerous in the nonbenzidin cells a faint brownish tinge in the region of masses of small carbon particles may sometimes be seen. This is seen also in smears not treated with benzidin as well as in the phagocytes of rabbit's blood, in which no benzidin reaction is present. This slight brownish discoloration is due to the finest ink particles and is readily distinguished from the coarser particles of benzidin reacting cytoplasm.

A second method has been employed to determine by direct microscopic examination the occurrence in the blood of neutral red reacting phagocytes that do not color with benzidin. Smears of the leukocytic layer (not treated with ink) supravitally stained (Method D) when dry were examined with oil immersion lens. The dye could be seen but was less distinct than in the fresh preparation. A cell or a small group of cells was placed at the center of a field under the oil immersion objective and then with the aid of low power lens a ring about the cell was scratched in the film with a teasing needle. The location of the cells "spotted" was noted and the preparation treated with benzidin (Method B) followed by hematoxylin. The red was dissolved by the alcoholic benzidin, but the heavier neutral red reacting masses in cells positive to benzidin colored a greenish tinge instead of the usual brown. The red was completely removed from the cytoplasm of cells that did not react to the benzidin. In five smears from one leukocytic layer sixteen mono-

nuclear cells containing heavy masses of neutral red (Fig. 2, *C* and *D*) were located and afterwards treated with benzidin. Four of the sixteen were devoid of brown coloring while the other twelve showed a heavy greenish staining with the benzidin. The statements of the general morphology of the two groups of phagocytes identified by the phagocytic and peroxydase methods apply to these two groups. The nuclei of the benzidin cells are more broken in outline and are covered with oxydase granules. They stain much like the nuclei of the polymorphonuclear neutrophils.

COMMENT AND SUMMARY

A procedure of first importance in obtaining information in regard to cells present in the blood in scant numbers is to concentrate them in a leukocytic layer with the least possible injury to the cells. The appearance in the peripheral blood of the hyaline type of leukocyte derived from the endothelium of the blood capillaries, the hemendotheliocyte, appears to be pathologic and this cell has been seen only in rabbits given huge doses of carbon suspension. The cell is identical with the hyaline type observed by me[9] in the peritoneal exudates. Since it is marked by the ink and contains little or no neutral red, this cell presents a characteristic appearance in supravital films in both blood and peritoneal exudates. The blood capillary type of endothelial cell, such as the Kupffer cell, is in these animals heavily marked with ink and reacts little to neutral red. The phagocytes anchored in the splenic sinuses, which have ingested enormous quantities of carbon, react little to the dye. The results obtained by vital staining with colloidal dyes, by marking the vascular endothelium with carbon in suspension and by supravital staining support the view that this hyaline type of phagocyte arises from the endothelium of the capillary blood vessels and sinuses. The only specific designation for such a cell appearing in the peripheral blood is that of the hemal type of endothelial leukocyte or the hemendotheliocyte.

The neutral red reacting cell, on the basis of information previously presented,[9] is considered to be a derivative of lymphatic reticulo-endothelium and may therefore be spoken of specifically as the endothelial leukocyte of lymphatic origin, or as the lymphendotheliocyte. The number of lymphendotheliocytes in the blood has been increased experimentally in two ways: first, in the rabbit by injection of whole rabbit's blood into the peritoneal cavity (maximum of 7 per cent. in case of Rabbit H 16), and, second, in the guinea-pig by infecting with *Bacillus tuberculosis* (maximum of 8.3 per cent. in a tuberculous guinea-pig, H 13). The association of these experimental lymphendotheliocyte increases with definite lymph node enlargement is significant. The groin

lymph nodes of Rabbit H 16 (table) measured 3 by 5 by 9 mm. and microscopically the reticulo-endothelium was much hypertrophied and rosettes of neutral red granules were present in the cytoplasm of some of the lining cells (Fig. 3, *A*). In the tubercles of guinea-pigs a beautiful rosette grouping of the granules was seen in the large epithelioid cells (Fig 3, *B*). Only the inguinal, iliac, cervical and tracheobronchial lymph nodes have been examined but it seems likely that wherever anastomosing lymph channels of a capillary type occur these phagocytes may arise. In the secondary lymph nodules of lymph nodes the neutral red reaction has been obtained only in the reticular cells at the periphery, but usually the dye has not penetrated in a satisfactory manner to the germinal centers. The lymphocytes in supravital films of blood often contain from one to several neutral red granules in a small focus of cytoplasm, but in the cellular "juice" expressed from lymph nodes on dry dye films the excess of dye is absorbed by the numerous cells and only the larger reticular cells show a focus of dye granules. Likewise, when the dye is injected into the lymph nodes for the preparation of paraffin sections, only the reticulo-endothelium contains the dye. Since the lymphoid tissue of the spleen is usually stated to contain reticulum this organ has been given special attention. Cunningham, Sabin and Doan [7] found from 15 to 20 per cent. of the cells obtained from the spleen under anesthesia by puncture with a fine capillary pipet to be of the rosette type. My experience [9] was that these cells were present in about the same proportion to the other cells that they were in the blood. In supravitally stained paraffin sections typical rosette cells have not been demonstrated in the splenic corpuscles. Splenectomy does not influence the number of the rosette type of cell appearing in peritoneal exudates. The phagocytic activity of the lymph-endotheliocytes appears to serve as a certain means of separating them from the lymphocytes, but it is not certain that both may not arise from a younger common stem cell, although no evidence has been obtained to support such origin.

The third type of mononuclear phagocyte appearing in peripheral blood reacts both to neutral red and to benzidin. Naegeli [10] named these cells monocytes and noted that they give the oxydase reaction. He did not, however, recognize any type of mononuclear phagocyte other than the monocyte. Since others, among whom are Schilling,[19] have found that cells of this general type did not give the oxydase reaction, it may again be pointed out that it is necessary to specify the particular form of this reaction employed. However, it is possible that

19. Schilling, V.: Ueber hochgradige Monozyten mit makrophagen bei Endocarditis und über die Herkunft der grossen Mononucleären, Ztschr. f. klin. Med. **88**:377, 1919.

Schilling, for example in his cases of infectious endocarditis, was dealing with lymphendotheliocytes or with hemendotheliocytes, both of which fail to react with benzidin. Human bone marrow obtained at necropsy has been examined by Method C. It has usually been expressed from the cut ends of the ribs by crushing with bone forceps and at times has been placed in the fixing fluid within one hour after death. In sections of tissue the reaction of the benzidin is fully as intense as in smears but the brown is diffuse rather than in the form of individual granules. The presence of numerous myeloblastic cells makes difficult the determination of a second type of benzidin reacting cell in marrow. There are always present a considerable number of cells reacting less intensely than the usual myelocyte and the nuclei of many of these have the irregularity of outline seen in the benzidin-positive mononuclear cells of the blood. In the splenic pulp I [13] previously observed the presence of cells that resembled the blood mononuclears more than they did the myelocytes.

That the spleen is not the sole source of these cells is shown by the examination of the blood of an adult, E. M., from the surgical service of Barnes Hospital, St. Louis, before and after splenectomy: Nov. 4, 1924, before splenectomy, there were 1.9 per cent. of benzidin-positive mononuclear cells; three days after splenectomy, 2.7 per cent.; eight days after splenectomy, 2.3 per cent. June 22, 1925, there were 4.3 per cent. of mononuclear cells reacting to benzidin. In all except the last examination there was a relative increase in polymorphonuclears and the white cells per cubic millimeter were 21,000, 27,000 and 22,000, respectively, on the three days on which the first three examinations were made. The clinical diagnosis was purpura.

Since these cells are demonstrable only in the bone marrow, spleen and blood stream it seems likely that they arise chiefly in the bone marrow and to a less extent in the splenic pulp. Their property of ingesting much carbon in citrated blood in which there is very little phagocytosis by the polymorphonuclears may be interpreted to mean that these cells are not of the usual myeloblastic series. The alternative explanation is that there is a stage in the differentiation of the myelocyte when the cell has an unusual affinity for carbon. A reaction to benzidin less intense than that of the neutrophilic myelocytes also suggests that they are a distinctive type of cell. It may prove that a close relationship exists between these and the myeloblastic series, and Evans [20] found neutrophilic leukocytoses and increases in these cells to be associated in some instances. The term monocyte proposed by

20. Evans, F. A.: Observations on the Origin and Status of the So-Called Transitional White Blood Cells, Arch. Int. Med. **18**:692 (Nov.) 1916.

Naegeli for this peroxydase-reacting mononuclear leukocyte should be applied to this particular cell alone and not to the nonbenzidin reacting phagocytes arising from endothelium.

CONCLUSIONS

1. Monocytes, mononuclear benzidin-positive phagocytes reacting to neutral red by the method of supravital staining, are present in normal human blood and an occasional one is present in the blood of the normal guinea-pig. These cells have not been demonstrated in rabbit's blood. They are increased in the blood of the guinea-pig during the terminal stage of tuberculosis. Since they are found only in the bone-marrow and spleen these fixed tissues are regarded as the probable source for their production.

2. Lymphendotheliocytes, benzidin-negative mononuclear phagocytes that by the method of supravital staining react to neutral red (sometimes with arrangement of the dye granules in the form of a rosette), are present in the peripheral blood of the normal guinea-pig and rabbit and in human blood. They are increased in the blood of the rabbit by intraperitoneal injections of whole rabbit's blood and in the guinea-pig's blood during tuberculous infection. They arise from the lymphatic reticulo-endothelium. These leukocytes become transformed into the epithelioid cells of tubercles with preservation of their characteristic reaction to neutral red.

3. Hemendotheliocytes, benzidin-negative mononuclear phagocytes not reacting by the method of supravital staining to neutral red or reacting slightly to neutral red with the appearance of irregular dye cytoplasmic granules, have not been demonstrated in the normal peripheral blood. They appear in the blood of the rabbit following intravenous injections of India ink. These phagocytes, which react earlier and more intensely to intravenous carbon in suspension and to colloidal dyes than do other cells, probably arise from the blood vascular endothelium.

4. By direct examination, therefore, three kinds of mononuclear phagocytes may be demonstrated in the circulating blood, two of which are found under normal conditions. Less direct evidence points to the conclusions that the benzidin-reacting phagocytes are derived from the bone marrow and the spleen, and that the two benzidin-negative phagocytes arise from the lymph nodes and from the endothelium of capillary blood vessels, respectively.

THE ETIOLOGY OF CHRONIC ULCERATIVE COLITIS

EXPERIMENTAL STUDIES WITH SUGGESTIONS FOR A MORE RATIONAL FORM OF TREATMENT *

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A year ago we believed we had isolated the organism responsible for at least a prominent part in the cause of chronic ulcerative colitis of the so-called idiopathic type.[1]

The organism was obtained with sterile swabs through the proctoscope from the depths of an ulcer of the colon, after the latter had been thoroughly cleansed by repeated washings and the top of the ulcer had been carefully wiped with a preliminary swab. Cultures of stools failed to yield the organism but in cultures of the rectal discharge of mucus, pus and blood it was often found as the vastly predominating bacterium. The material obtained was cultured immediately in warm dextrose brain broth,[2] blood agar and lactose agar and incubated for twenty-four hours. All mediums were titrated to a p_H 7.4. The organism isolated in this way as the predominating bacterium and often in pure cultures is a gram-positive, lancet shaped diplococcus, growing in twos and fours (Fig. 1) and has been observed with a capsule occasionally, thus morphologically resembling a pneumococcus. It is not bile soluble. Twenty strains examined have failed to ferment inulin or mannite but have always fermented dextrose, lactose, saccharose, maltose, raffinose, salicin and acidified milk, shortly after their isolation. When retested weeks or months after isolation, the fermentation of the last six sugars has varied. The diagnostic pneumococcus serums of Types I, II and III have failed to agglutinate a number of typical strains. On blood agar the diplococcus grows as an alpha-hemolytic streptococcus (Fig. 2). Agglutinins and precipitins have been demonstrated in the blood of rabbits immunized with various strains of this organism. Cross agglutination and precipitation have been found among at least six strains examined.

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* From the division of medicine, Mayo Clinic.

* Read before the Section on Pharmacology and Therapeutics at the Seventy-Sixth Annual Session of the American Medical Association, Atlantic City, N. J., May, 1925.

1. Bargen, J. A.: Experimental Studies on the Etiology of Chronic Ulcerative Colitis, J. A. M. A. **83**:332-336 (Aug. 2) 1924.

2. Rosenow, E. C.: Studies on Elective Localization, Focal Infection with Special Reference to Oral Sepsis, J. Dent. Res. **1**:205-268, 1919.

In two years, cultures have been made from the lesions of sixty-eight patients with chronic ulcerative colitis. In 80 per cent. of the cases, such a diplococcus has been isolated.

Cultures also have been made by swabbing the healthy colon mucosa of twenty persons presenting themselves for proctoscopic examination because of hemorrhoids or other rectal complaints, and only once were diplococci of similar characteristics isolated.

Smears of the material obtained from the ulcers consist of pus cells and red blood cells but, in the majority of them, diplococci of similar structure can be recognized in appreciable numbers.

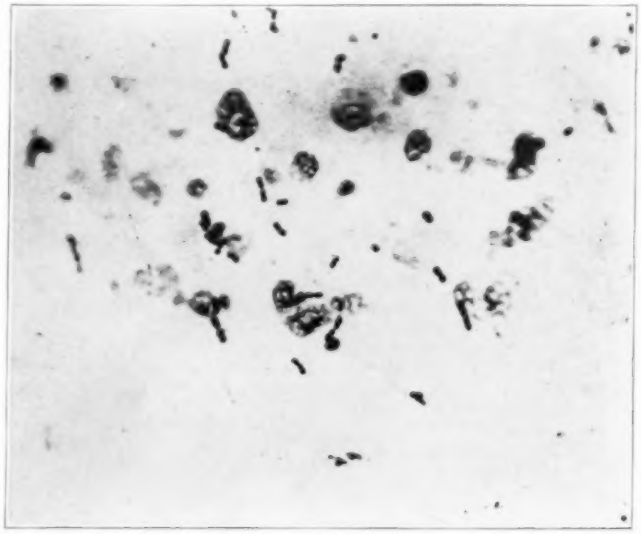

Fig. 1.—Freshly isolated culture of the diplococcus described.

Since our last report 139 rabbits have been injected intravenously with dextrose brain broth cultures in amounts varying from 5 to 15 c.c. Lesions developed in the colon in forty-five of these without lesions being grossly demonstrable elsewhere. Fully as many developed diarrhea; these rabbits were injected with pure cultures of rapidly isolated diplococci. Rosenow and his co-workers, using a similar technic, have injected large numbers of rabbits with various strains of streptococci and have never found lesions in the colon such as these.

The organism sometimes localizes in the gallbladder of rabbits. Eighteen rabbits were fed on vitamin-free food (crushed oats and water or autoclaved rice and water, according to the methods of McCarri-

son [3]) for two weeks. At the end of this time, nine were injected intravenously with 5 c.c. of a dextrose brain broth culture of the freshly isolated diplococcus. All were dead within five days; eight had lesions of the colon, from submucous hemorrhages to minute ulcers. Three of the eight had empyema of the gallbladder from which a pure culture of the diplococcus was isolated.

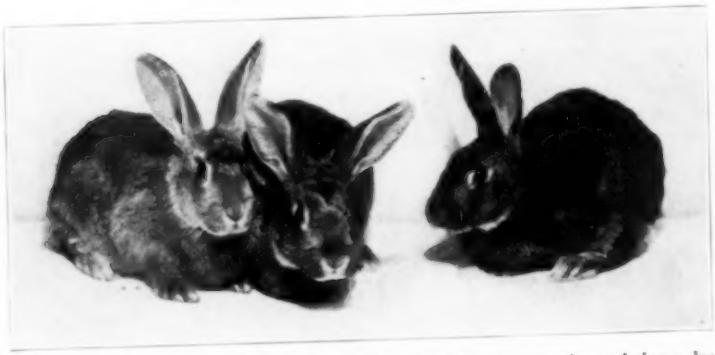

Fig. 3.—Three rabbits fed on vitamin-free food for two weeks and three days.

Fig. 4.—Three rabbits three days after injection with the diplococcus fed on vitamin-free food for three weeks.

Of the other nine, five died, apparently from starvation with certain of the symptoms of a deficiency syndrome, while four lived two months on the diet (Figs. 3 and 4).

3. McCarrison, Robert: Studies in Deficiency Disease, London, Frowde, Hodder and Stoughton, 1921.

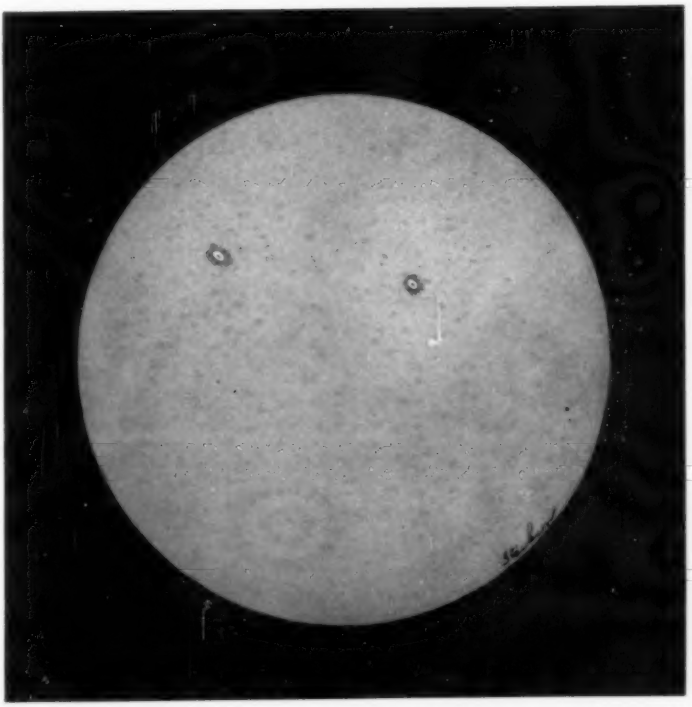

Fig. 2.—Typical twenty-four hour colonies isolated from acute lesions of the colon in pure culture.

The culture of the diplococcus isolated from these gallbladders was injected intravenously into two young dogs, weighing 4 and 6 kg., respectively. Repeated daily injections were given in 12, 20, 20, 20, 20, and 30 c.c. in an effort to produce a chronic disease more like that in human beings, since the lesions in rabbits were rather of the acute fulminating type. On the fourth day the dogs began passing bloody mucus; on the sixth there was marked rectal prolapse exposing an edematous, granular, easily bleeding, superficially ulcerated mucosa, the typical picture of early ulcerative colitis. At the end of two weeks, one of the dogs began to improve, and repeated large doses of the organism had no effect. When it was killed at the end of three weeks, a healing ulcerative colitis of the lower 6 inches (15.2 cm.) of the colon was found. In the other dog colon changes were demonstrable by roentgen ray with the barium enema at the end of three weeks, after which he too began to get well (Fig. 5).

The possibility that gallbladders might act as a focus for harboring these diplococci made it seem reasonable that other distant foci in human beings might be of importance.

A woman, aged 26, entered the Mayo Clinic, Dec. 30, 1924, with a history of having had bloody diarrhea for three years. Eight months previous to her first spell of diarrhea, she had had a tooth devitalized and it had given her some trouble ever since. In the autumn of 1921, she had diarrhea for two weeks, without apparent cause. In the spring of 1922, she had diarrhea for six weeks. In the spring of 1923, her diarrhea recurred and lasted until her admission to the clinic. From twelve to twenty stools a day were often passed, with mucus, blood and pus. Chronic ulcerative colitis was discovered; a diplococcus was isolated from the rectal lesions, and a vaccine prepared for her. When 0.1 c.c. of it was given subcutaneously there was a marked increase in the diarrhea. Several days later 0.2 c.c. was given with an even more severe diarrhea and marked soreness of her dead tooth in which a periapical abscess was revealed by the roentgen ray. The vaccine was then discontinued and her septic tonsils and, later, her abscessed tooth were removed. The extraction was followed by a severe upset of the bowel. In the culture of the periapical abscess, diplococci of the variety described predominated in large numbers, with short chain streptococci making up the remainder of the culture.

The six hour glucose brain broth subculture of the twelve hour culture of the pus from this periapical abscess was injected intravenously into a rabbit, and in twenty-four hours the animal was dead. Extensive submucous hemorrhages and minute ulcers of the lower three-fourths of the colon were found (Fig. 6). A pure culture of the diplococcus isolated from the mesenteric lymph nodes, which were very

large and acutely hemorrhagic, was injected into two young dogs, weighing 7.2 and 11 kg., respectively. Both were dead in less than twenty-four hours, the floor of the cage being covered with blood. Massive submucous hemorrhages were found in the colon and disseminated hemorrhages in the lower ileum and in the upper jejunum

Fig. 5.—Dog's colon showing ulcerative colitis by barium enema three weeks after initial injection of diplococci.

(Fig. 7). In neither the rabbit nor the dogs were lesions found elsewhere.

Since this striking result we have seen repeated severe bowel reactions with acute bloody dysentery, following extraction of such foci as teeth and tonsils. One young woman after a series of injections of

vaccine filtrate was doing well, with one or two stools daily and no blood, when her septic tonsils were removed. Several days later, following the administration of 0.6 c.c. of vaccine, she had a terrific reaction with fifteen bloody stools in twenty-four hours and marked prostration. After she had rested a week in bed and her bowels had gradually quieted, 0.3 c.c. of vaccine was administered, the dose being increased by 0.1 c.c. every third day. In two weeks she felt entirely well, and had one formed movement a day. She has remained well.

These facts clearly demonstrate the importance of distant foci in the etiology of this disease. How important they may be in its continu-

Fig. 6.—Extensive submucous hemorrhages and minute ulcers of lower three-fourths of colon of rabbit injected intravenously with organism obtained from periapical abscess.

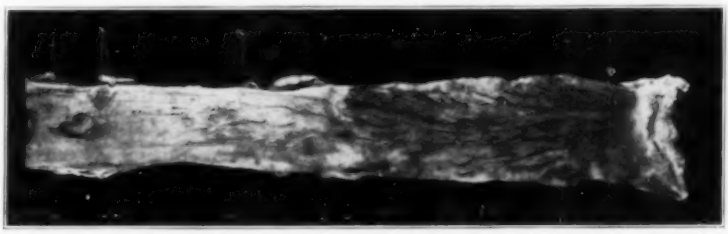

Fig. 7.—Massive submucous hemorrhage of dog's colon injected with pure culture from mesenteric glands of the same rabbit.

ance, it is difficult to say, but their part in the early stages certainly seems established.

Lesions observed in their early stages have appeared as miliary abscesses (Fig. 7). A few days later superficial ulceration of the tops of the abscesses and their confluence produce the typical granular appearance. From such lesions, seen on the protruding bowel after ileostomy, a pure culture of the diplococcus has been isolated in the minute drop of pus obtained from the tiny abscesses in the mucosa. These repeated "showers" of infection and healing give one a clear

conception of how the thick layer of granulation tissue and underlying fibrous tissue may be formed, as it is seen in cross sections of the colons of these patients after death. In sections through the lesions stained for bacteria, large numbers of gram-positive diplococci of a structure similar to those described are commonly seen (Fig. 8).

Fig. 8.—Five and six day old lesions of protruding portion of intestine after ileostomy in case of chronic ulcerative colitis.

With these facts before us, it would seem logical that some form of immunization against this diplococcus offers hope for patients suffering from chronic ulcerative colitis. This has been attempted by preparing vaccines and vaccine filtrates according to the methods of Rosenow. Vaccines are prepared by heavily inoculating 8 ounce (236 c.c.) bottles

of 1 per cent. dextrose broth, incubating for twenty-four hours, centrifugating and making a thick glycerin suspension of the bacterial precipitate. To 24 c.c. of sterile physiologic sodium chlorid solution is added 0.2 c.c. of the glycerin suspension and 0.08 c.c. of tricreosote, making a suspension of 2,000,000,000 dead bacteria for each cubic centimeter. This is allowed to stand in the icebox over night. Vaccine filtrates are prepared by passing the original dextrose broth culture through a Berkefeld filter and adding 0.04 c.c. of tricreosote to the sterile filtrate.

Of either material, 0.1 c.c. is given as the initial dose. Patients who had slight systemic reaction with the small doses have obtained the best results. Recently we have used more filtrates, seemingly with better results. Injections are made subcutaneously every third day, beginning with 0.1 c.c., the dose being increased by 0.1 c.c. at each injection up to 1 or 1.5 c.c. according to the patient's reaction to the toxin. In two or three weeks, the diarrhea subsides and the patients improve, often very rapidly. Twelve patients have been dismissed clinically well and, on proctoscopic examination, at least much better; nine have improved; others have either not taken the vaccine or filtrate, or not enough of it to justify a statement with regard to results (Tables 1 and 2).

In conjunction with the vaccine treatment, we have, of course, continued our usual treatment of the disease. When the lesions are low enough to be reached by proctoscope, local treatment with 1:4,000 aciflavin, 1 per cent. mercurochrome, silver nitrate, lunar caustic and similar medicaments have been applied. Irrigations with hot water, warm physiologic solutions of sodium chlorid, argyrol, acriflavin 1:4,000, and instillations of witch-hazel and bismuth in olive oil also have been used. An important adjunct to the treatment is the administration of tincture of iodin by mouth, according to the methods of one of us,[4] and kaolin in doses of from 1 to 1.5 ounces. The nature of the action of the former is not definitely known, but a leukocytosis is often produced by its use. The kaolin presumably acts as an adsorbent and may have an antiperistaltic action. We have allowed these patients a rather generous diet, avoiding only such food as they themselves have found to upset their bowels.

SUMMARY

A gram-positive diplococcus has been isolated in 80 per cent. of cases of chronic ulcerative colitis. This organism has been obtained in pure culture from the early lesions, in an acute exacerbation of the disease, from the depths of chronic ulcers, and occasionally from distant

4. Logan, A. H.: Three Cases of Ulcerative Colitis Cured with Iodin, Med. Clin. N. A. **7**:105-112 (July) 1923.

TABLE 1.—*Patients with Chronic Ulcerative Colitis Treated with Vaccines and Vaccine Filtrates*

Patients Clinically Well on Dismissal *

Case	Sex	Age, Years	Objectively Well, Months	Symptoms Previous to Admission	Roentgen-Ray Findings in Colon on Admission	Proctoscopic Examination	
						On Admission	On Dismissal
1	F	31	7	5 months	Chronic ulcerative colitis	Chronic ulcerative colitis	Well
2	F	57	5	12 years	Chronic ulcerative colitis of recto-sigmoid	Chronic ulcerative colitis	Improved 30 per cent.
3	M	43	13	2 years	Negative	Chronic ulcerative colitis	Improved 50 per cent.
4	F	30	8	6 years	Chronic ulcerative colitis of left half of colon	Chronic ulcerative colitis	Improved 75 per cent.
5	M	40	4	17 months	Chronic ulcerative colitis of entire colon	Chronic ulcerative colitis	Improved 30 per cent.
6	M	..	9	1 year	Doubtful	Chronic ulcerative colitis	Well
7	M	29	3	7 years	Negative	Chronic ulcerative colitis	Well
8	F	25	2	7 years	Chronic ulcerative colitis of left half of colon	Chronic ulcerative colitis	Improved 30 per cent.
9	F	36	1	3.5 years	Negative	Chronic ulcerative colitis	Well
10	F	28	2	3 years	Chronic ulcerative colitis of recto-sigmoid	Chronic ulcerative colitis	Improved 50 per cent.
11	F	16	2	3 months	Negative	Chronic ulcerative colitis	Well
12	F	40	3	6 years	Negative	Chronic ulcerative proctitis	Practically well

* One well-formed stool daily.

TABLE 2.—*Patients with Chronic Ulcerative Colitis Treated with Vaccines and Vaccine Filtrates*

Patients Clinically Improved on Dismissal

Case	Objectively Well, Months	Duration of Symptoms	Roentgen-Ray Findings in Colon on Admission	Proctoscopic Examination	
				On Admission	On Dismissal
1	3	6 years	Chronic ulcerative colitis of left half of colon	Chronic ulcerative colitis	Slight improvement
2	12	25 months	Ulcerative colitis of sigmoid	Chronic ulcerative colitis	Improved 50 per cent.
3	4	11 years	Chronic ulcerative colitis of entire colon	Chronic ulcerative colitis 3+	Improved 50 per cent.
4	2	1 year	Chronic ulcerative colitis	Chronic ulcerative colitis 2+	Slight improvement
5	2	20 months	Chronic ulcerative colitis	Anal ulcers	
6	2	16 years	Chronic ulcerative colitis of entire colon	Chronic ulcerative colitis with marked contraction	Improved
7	2	4 years	Chronic ulcerative colitis	Chronic ulcerative colitis 3	Improved
8	3	1 year	Negative	Granular proctosigmoiditis	Improved 25 per cent.
9	2	10 years	Negative	Chronic ulcerative proctitis	Improved 50 per cent.

foci elsewhere in the body. If searched for at the proper time when the lesions are active, we believe that the organism would always be found. By the intravenous injection of the organism, lesions like those in human beings have been produced in rabbits and dogs, the lesions in the latter approaching the chronic stage of the disease in human beings. The finding of this organism in distant foci and the production of acute lesions in the colon could account for the repeated exacerbations of the disease and the pathologic findings of layer on layer of the granulation and fibrous tissue, replacing the colon mucosa.

From these studies, it would seem that the hope of control of this disease lies in (1) removal of the distant foci of infection; (2) immunization in some form against the causative organisms; (3) local and topical application and irrigation; (4) the empiric use of drugs, such as tincture of iodin; (5) the administration of some adsorbent like kaolin by mouth, and (6) a nonirritating yet general diet.

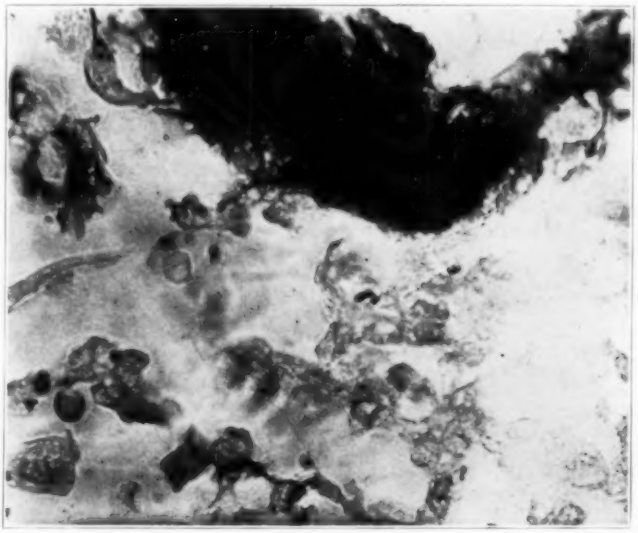

Fig. 9.—Section through lesion of colon of chronic ulcerative colitis, showing diplococci.

REPORT OF CASES

CASE 1.—A woman, aged 31, came to the clinic, May 19, 1924, with a history of having had bloody diarrhea with mucus and pus during the last year. She had had from fifteen to forty stools in twenty-four hours with severe abdominal cramps and rectal tenesmus. Except for two weeks of freedom, two months before coming to the clinic, her trouble had steadily progressed with loss of 28 pounds (12.7 kg.) in weight.

A very diffuse, active ulcerative proctosigmoiditis, with activity 3 on a basis of 4, was found by proctoscopic examination. The roentgen ray demonstrated that the disease included the transverse colon. Repeated examinations of the stool failed to reveal parasites or the bacilli of tuberculosis, but gram-positive diplococci were isolated by culture. Because of the patient's acute condition, she was admitted to the hospital and given daily hot water irrigations (at 120 degrees F.), argyrol instillations, kaolin and tincture of iodin by mouth and autogenous vaccine. The vaccine treatment was continued for four months. She reported, Jan. 7, 1925, for reexamination. By proctoscopic examination the bowel was entirely healed. The roentgenogram of the colon was negative. She had one well formed stool daily and was clinically well.

Case 2.—A woman, aged 37, came to the clinic, Nov. 24, 1924, with a history of rectal bleeding which had become progressively worse during the last twelve years, so that during the last six months she had lost 25 pounds (11.3 kg.), and was passing six or seven stools with much blood, mucus and pus daily.

The proctoscopic examination revealed diffuse inflammation of the rectum and sigmoid, contraction to one-third of their usual lumen, and irregular ulcers with membranous bases and bleeding margins. The roentgen ray revealed a large dilated colon with a lesion of the sigmoid. A diagnosis of chronic ulcerative colitis was made. Repeated examinations of the stool failed to reveal the bacilli of tuberculosis or amebas, but cultures of the rectal discharge yielded a large predominance of gram-positive diplococci. The patient had slight anemia; the leukocytes numbered 10,100. She was given autogenous vaccine, local topical applications of witch-hazel, 15 per cent. silver nitrate solution, lactose three times daily, and tincture of iodin by mouth. Dec. 18, 1924, she left the clinic showing 30 per cent. improvement on proctoscopic examination. She wrote after two months that she was entirely well, and a recent report was similar in tone.

Case 3.—A man, aged 43, came the clinic in May, 1922, with a history of bloody diarrhea with mucus and pus for two months. He had marked urgency and tenesmus with passage.

Repeated tests of the stool did not reveal parasites or acid-fast bacilli. Roentgenograms revealed disease of the rectosigmoid and median pelvic colon. After proctoscopic examination, chronic ulcerative colitis was diagnosed. The patient was given the usual local treatment and emetin hydrochlorid hypodermically, and improved slightly. He then returned at intervals of several months with acute exacerbations and early in 1924 a gram-positive diplococcus was isolated by culture. A vaccine was prepared and administered. The usual local treatment was continued, namely, instillations of witch-hazel, with irrigations of hot water and applications of lunar caustic. When seen in August, 1924, he was entirely well clinically and the proctoscope showed healed lesions. He has remained well to this time.

Case 4.—A woman, aged 28, came to the clinic because she had been troubled for four years with intermittent bloody diarrhea, up to twenty stools daily, with much mucus, pus and blood. The first spell lasted two weeks, the next six weeks, and the last continued for one and a half years, until the time of admission.

Repeated tests of the stool failed to show the presence of parasites, but from the scrapings from the lesions a predominance of gram-positive diplococci was cultured. Roentgen-ray examination of the colon revealed a lesion of the rectosigmoid suggestive of ulcerative colitis, and after proctoscopic examination a diagnosis of ulcerative colitis was made. The patient also had a chronic polyarthritis of several years' standing. One tooth with a periapical abscess and two impacted molars were discovered; the condition of the tonsils was questionable.

With the pus of the periapical abscess, lesions were produced in rabbits. A marked bowel reaction followed the extraction of the teeth, but soon afterward improvement of the arthritis and colitis was manifested. She was given a course of vaccine, at first with a violent reaction even with 0.1 c.c., but the reactions gradually diminished. She was given local treatments with 15 per cent. silver

nitrate solution, lunar caustic and witch-hazel. On dismissal the proctoscope showed healed lesions and she had recovered clinically from her arthritis and colitis.

CASE 5.—A man, aged 29, came to the clinic, Feb. 4, 1925, because of persistent passage of blood and pus by rectum since his discharge from the army in 1918. He had difficulty in passing stools, but he had had from three to five passages of blood and pus daily. In two years he had lost only 6 pounds (2.7 kg.) and ate well. His only other symptoms were severe distress from gas and frequent rectal spasms.

On proctoscopic examination a granular, easily bleeding mucous membrane of the colon was found, covered by much exudate. A diagnosis of proctosigmoiditis was made. The patient was placed on an anticonstipation regimen, had daily local applications of 15 per cent. silver nitrate solution, alternating with 3 per cent. mercurochrome or lunar caustic, with instillations of witch-hazel and a series of injections of vaccine filtrate. March 11, 1925, he was dismissed clinically well and the proctoscope revealed healed lesions. He was advised to continue the vaccine treatment and to have both infected tonsils removed at home.

CASE 6.—A man, aged 40, came to the clinic in January, 1924, because of four months of bloody diarrhea of very gradual onset. During the last three months he had been passing ten or more stools a day with blood, pus and mucus, with much tenesmus. He had lost only 9 pounds (4.1 kg.), and except for his abdominal distress had no complaints.

A diagnosis of chronic ulcerative colitis was made after proctoscopic and roentgenographic examinations with the barium enemas. The entire colon was found to be affected. Periapical abscesses were found around two teeth.

The patient was sent home for a month under treatment with tincture of iodin and kaolin, and advised to have his infected teeth extracted. He returned in a month complaining of only two or three movements daily, and on proctoscopic examination he was 85 per cent. improved. He went home and for the next five months passed only two or three stools a day with very little blood, but in July, with an acute exacerbation, he had from eight to fifteen stools daily with much blood and pus. After some improvement, he returned home but without much relief, and at a visit in September he also had a severe myositis. In October, 1924, a colostomy was performed. Slight improvement followed, but he returned the first week in March, 1925, and was given a severe course of vaccine treatment. He was dismissed the last of the month with a permanent colostomy. He was found to be in good condition clinically and the colon was healed proctoscopically.

SPINDLE CELL SARCOMA OF THE HEART *

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Tumors of the heart, although observed by the early anatomists, are of uncommon occurrence. In 1685 Zollicofferus [1] wrote a dissertation on the subject, "De Polypo Cordis," and in 1700 Boneti [2] noted an intracardiac polyp attached by multiple roots to the wall of the right ventricle.

There are in the literature about 150 case reports of neoplasm primary in the heart, but reviews of the subject by different writers do not agree in the number that is authentic. Eighty-six [3] and thirty [4] cases, respectively, were compiled in 1893, and forty-six [5] and ninety-one [6] in 1908. The discrepancy in these numbers is due to the question in each recorded case whether the tumor had its origin in the heart.

As indicated by the foregoing reports the most frequent of the primary tumors of the heart are fibromas, myxomas and fibromyxomas. Rhabdomyomas, lipomas, angiomas, lymphangiomas and teratomas are rare. It is maintained by several investigators that many tumors reported as fibromas or fibromyxomas are thrombi that have become organized.

Thirty-one cases of sarcoma primary in the heart were compiled by Perlstein [7] in 1918. To this may be added three cases that have been reported during the interim.[8]

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1. Zollicofferus, quoted by Morgagni: De Sedibus et Causis Morborum, Epist. Anat. Med. Article 30, **24**:431.

2. Boneti, Theophili: Sepulchretum sive Anatomia Practica ex Cadaveribus Morbo Denatis, Lib. IV, Sect. 12, Observatio 19, p. 567, Tome I, Geneva, 1700.

3. Tedeschi, A.: Beitrag zum Studium der Herz-geschwülste, Prag. med. Wchnschr. **18**:121, 1893.

4. Berthenson, Leo: Zur Frage von der Diagnose primärer Neoplasmen des Herzens, Myxom des linken Vorhofes, Arch. f. path. Anat. u. Physiol. u. f. klin. Med. **132**:390, 1893.

5. Hagedorn, Oswald: Ueber primäre Herztumor, Centralbl. f. allg. Path. u. path. Anat. **19**:825, 1908.

6. Link, Richard: Die Klinik der primären Neubildungen des Herzens, Ztschr. f. klin. Med. **67**:272, 1909.

7. Perlstein, I.: Sarcoma of the Heart, Am. J. M. Sc. **156**:214 (Aug.) 1918.

8. Göttel, L.: Fall von primären Herzsarkom, Deutsch. med. Wchnschr. **45**: 1038, 1919. Goldstein, H. I.: Tumors of the Heart with a Report of Ten Cases of Cardiac Tumor, New York M. J. **115**:97; 158, 1922. Penault, L. G.: A Case of Primary Sarcoma of the Heart, Canad. M. A. J. **13**:108, 1923.

Neoplasms have been found in any of the four chambers of the heart originating from either endocardium, myocardium, epicardium or parietal pericardium. In the ninety-one cases tabulated by Link,[6] the localization was as follows: right auricle, ten; left auricle, twenty-four; right ventricle, fourteen; left ventricle, eight; valve tumors, sixteen; both auricles, three; both ventricles, two; right auricle and right ventricle, two; left auricle and left ventricle, two, and interauricular septum, two.

Analyses of the reported cases by several writers have led to no definite clinical picture diagnostic of the condition. The clinical picture varies with the location and the nature of the neoplasm, and these conditions are so variable that, according to Perlstein,[7] a neoplasm of the heart has never been diagnosed clinically. The location of the tumor may determine the competency of a valve, the integrity of the conducting mechanism or the patency of a coronary or pulmonary vessel; the size of the tumor may modify the functional condition of the myocardium or the capacity of a chamber to contain blood. A small sessile tumor may produce no symptoms; a pedunculated tumor may act like a ball-valve, and a rapidly growing tumor may lead to a very rapid disintegration of the cardiac mechanism. The contour of the heart may be distorted, and a tumor involving the epicardium may produce hemorrhage into the pericardial cavity. When present, metastases establish the diagnosis of malignancy.

In the case here reported the clinical diagnosis of sarcomatosis was made by the presence of disseminated sarcoma nodules. Involvement of the heart with sarcoma was suggested by the subnormal blood pressure, rapid pulse rate, systolic murmur, and an abnormal contour of the heart as seen in the roentgenogram. This was considered as a possibility but a definite diagnosis of sarcoma primary in the heart was not made clinically.

REPORT OF CASE

History.—A Russian man, aged 27, whose general health had been good, had as the first symptoms of illness an attack of nausea, vomiting and abdominal cramps, which occurred four months before death. These symptoms disappeared after a few days. About three weeks later, the patient noticed for the first time several painful nodules developing in the legs, the arms and the buttocks. The appearance of the nodules was followed by another attack of abdominal pain and this was accompanied with diarrhea, which lasted a few days. The physician attending the patient during that attack found the abdomen distended and rigid; the temperature, 38 C.; the white blood count, 4,000; the stool negative for blood, parasites and ova, and the blood Wassermann reaction negative.

The patient was admitted to Lakeside Hospital, Sept. 28, 1924, with the complaint of painful nodules in the buttocks, scrotum, legs and arms.

Physical Examination.—The patient was cachectic and presented marked pallor and dehydration. The temperature by mouth was 39 C. About thirty-five firm, discrete, well circumscribed nodules could be palpated in the muscles or in the subcutaneous tissue of the buttocks, scrotum, lower legs, right thigh, left upper

arm and chest. The nodules varied from a few millimeters to 1.5 cm. in diameter. Several nodules in the buttocks, scrotum, and chest elevated the skin. The larger nodules were painful to palpation. There was no general glandular enlargement. The neurologic examination was negative. The lungs were negative. The respiratory rate was 22 per minute. There was no marked precordial pulsation and no thrill. The heart sounds were distinct. A soft systolic murmur was heard over

Fig. 1.—Chest, showing a prominence in the region of the left auricle. The cardiopericardial shadow suggested the probability of an increased amount of fluid in the pericardial cavity.

the precordium of the same intensity and quality at the base as at the apex. The first sound was clear. The systolic blood pressure was 96, the diastolic 56. The pulse rate was 120, and the rhythm was regular. The abdomen was moderately distended, tympanitic, and contained a small amount of free fluid. There was tenderness to deep palpation in the left lower quadrant. A mass of indefinite outline was palpated in the hypogastrium. A rectal examination was normal.

Laboratory Findings.—The urine was normal. The blood examination showed: hemoglobin, 60 per cent.; red blood cells, 2,887,500, with no nucleated red cells; white blood cells, 15,400, with differential count of polymorphonuclears, 77 per cent.; mononuclears, 17 per cent.; transitionals, 5 per cent.; neutrophil myelocytes, 1 per cent. The vital capacity was 3,750 c.c. in a sitting posture; the expected capacity, 4,600 c.c. A blood culture was negative.

Fig. 2.—Section of a metastatic nodule removed from the deltoid muscle, showing spindle cell sarcoma.

A roentgenogram of the chest showed a well marked prominence in the region of the left auricle and pulmonary artery and a cardiopericardial shadow rather globular in shape, suggesting an increased amount of fluid in the pericardial cavity (Fig. 1).

The nodules described above were considered metastatic and sarcomatous. A small tumor removed from the right deltoid region was a spindle cell sarcoma (Fig. 2).

During the third day in the hospital the patient developed generalized peritonitis. The abdominal distention increased; the left lower quandrant became painful and rigid to palpation, and the temperature rose to 40.5 C. Death occurred on the seventh day after admission to the hospital.

The diagnosis was generalized sarcomatosis of undetermined origin, with metastases involving: (1) the intestine, producing a perforation and peritonitis, and (2) the muscles and subcutaneous tissue of the legs, buttocks, etc.

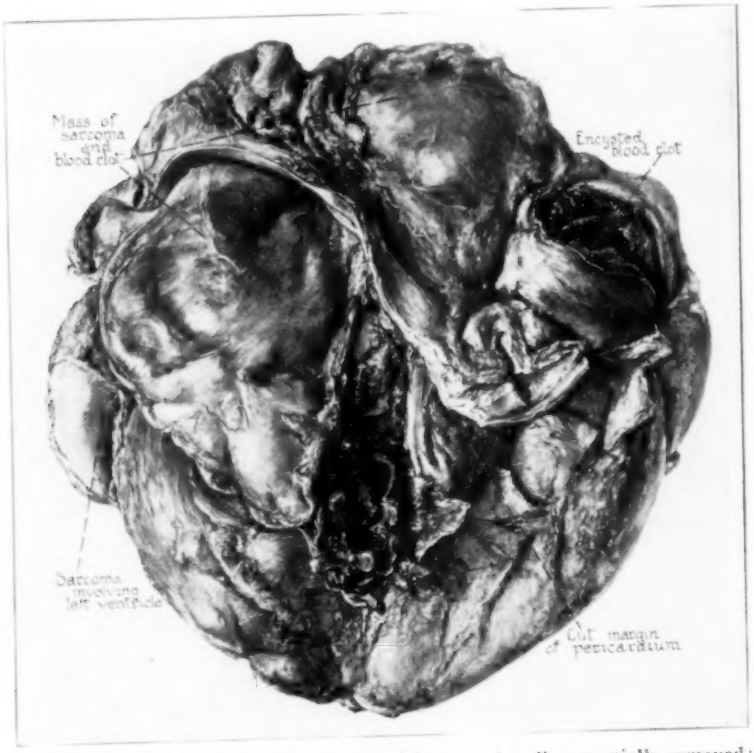

Fig. 3.—Posterior aspect of sarcoma with the pericardium partially removed; multinodular mass of sarcoma and blood clot over base of heart was covered by pericardium and the latter was not perforated by the tumor. An encysted mass of blood clot is shown to the right. The pericardial cavity contained about 250 c.c. of free blood.

Necropsy.—The metastatic nodules of the subcutaneous tissues and muscles described above were spindle cell sarcomas. There was no fluid in the chest, and no pleural adhesions were present. The pleura of the diaphragmatic, medial and posterior aspects of each lung was studded with firm, grayish white sarcomatous nodules varying in size from 0.5 to 2 mm. in diameter. Sarcoma was not found elsewhere in the lungs. The heart and pericardium weighed 1,100 gm. The pericardial cavity contained about 250 c.c. of free blood, part of which was coagulated. Posteriorly between the heart and the pericardium was a multinodular tumor of which the greatest thickness was about 5 cm., and which extended over the entire base of the heart and partially over the veptricles (Fig. 3). The

pericardium was adherent to the tumor, but its continuity was not broken. The mass in the pericardial cavity posteriorly consisted chiefly of encysted blood clot which was partially organized. The tumor at the base was an extensive sarcoma of the left auricle, several centimeters thick, displacing the pulmonary artery and its branches markedly upward, and extending through the endocardium between the entrance of the pulmonary veins. Here there were two intracavitary tumors (Fig. 4). One was a pedunculated tumor, the center of which contained sarcoma, measuring about 3 by 4 cm. over the surface, and about 2.5 cm. to the attachment

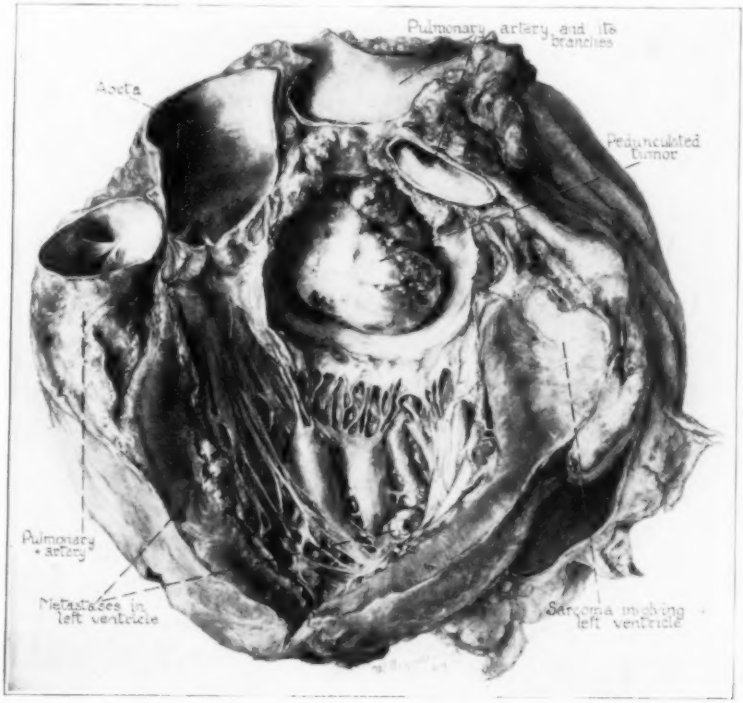

Fig. 4.—Extensive sarcoma involving the wall of the left auricle, displacing the pulmonary artery and its branches upward, extending into the cavity of the left auricle and into the wall of the left ventricle. Two metastases are present in the ventricle. The larger of the intracavitary tumors is a thrombus attached to the auricular wall by a pedicle that contains sarcoma. Lying above and behind the thrombus and partially obscured from view is a friable sarcoma which, undoubtedly, was the source of metastatic emboli. The pulmonary veins open on either side of these tumors and cannot be seen.

of the pedicle. It was firm, distinctly laminated, and in the outer portion consisted of dense fibrous tissue containing few nuclei. Sarcoma was present in the center of the tumor and in the pedicle. The tumor was not sufficiently free to act as a ball-valve at the mitral ring but, undoubtedly, it offered some obstruction to the filling of the left ventricle. The other intracavitary tumor was a lobulated sarcoma, about 2 cm. in diameter, and partially hidden by the pedunculated tumor (Fig. 5). This tumor was friable and, undoubtedly, was the source of multiple

sarcomatous emboli which were borne by the arterial blood. Anteriorly, the sarcoma extended over the left ventricle about 6 cm. from the mitral ring, and involved the epicardium and the outer portion of the myocardium. Two metastases, about 0.5 cm. in diameter, were present in the left ventricular wall near the septum and 3 cm. from the apex. The right ventricle and the right auricle were not involved.

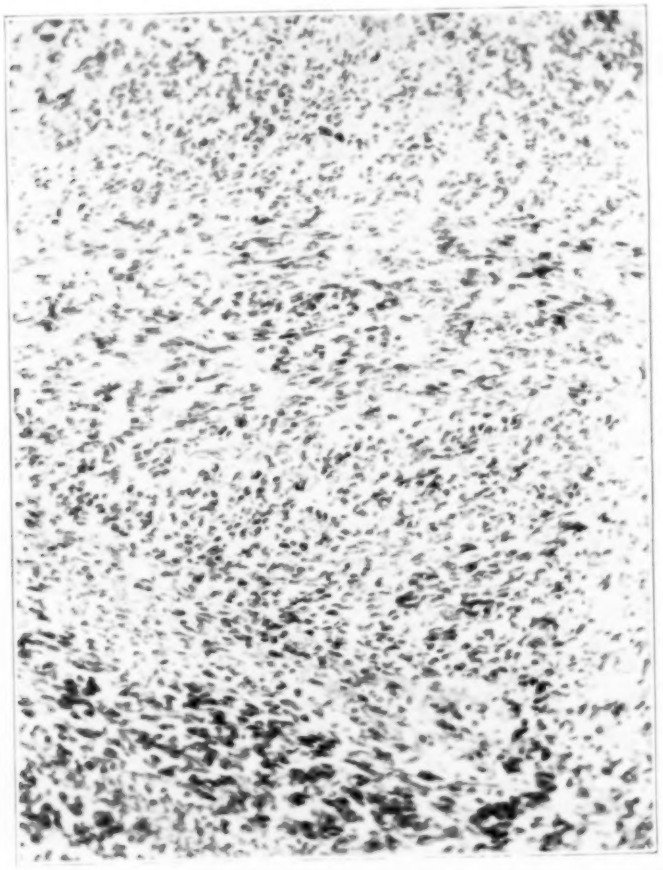

Fig. 5.—Section of the tumor of the heart showing spindle cell sarcoma.

The abdominal cavity contained about 500 c.c. of fluid stained with fecal material and green pus. Numerous nodules, about 2 mm. in diameter, were present in the parietal peritoneum. The intestines were bound together by fibrinous adhesions. A soft irregular mass, about 17 cm. in its greatest dimension, was found in the left upper quadrant binding together the ileum and the transverse and descending colon. In the center of this tumor was a perforation into the ileum. Numerous tumors were found elsewhere involving the various coats of the intestines and the stomach, varying from 0.5 to 11 cm. in diameter. Sarcoma was not found in the liver or brain.

COMMENT

Clinically, the presence of widely scattered nodules with the history and signs of intestinal obstruction and perforation indicated the diagnosis of sarcomatosis with metastases distributed by the arterial blood stream. Involvement of the heart was suggested by the presence of a murmur, low blood pressure, and a distortion in the cardiopericardial roentgenogram. Assuming that one pathologic condition should be held accountable for the entire clinical picture, we made the diagnosis of sarcoma involving the intestines, and the muscles and subcutaneous tissues.

The primary growth was, undoubtedly, in the wall of the left auricle. The distribution of the metastases indicated that they were borne by the arterial blood stream. The absence of metastases in the liver and lungs excludes the tumors of the intestine as the primary focus. The thrombus in the left auricle formed on the sarcoma that had involved the endocardium.

QUINIDIN IN THE TREATMENT OF AURICULAR FIBRILLATION, ESTABLISHED, PAROXYSMAL AND TRANSIENT *

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In spite of the fact that quinidin has been widely used over a period of six years in the treatment of auricular fibrillation, there still exists a difference of opinion concerning its indications and contraindications, as well as its true value and dangers. It therefore seems worth while to add to the already existing reports of its use, so that more material may be available for determining our final opinion regarding the usefulness of this drug in complete arrhythmia.

TYPES OF AURICULAR FIBRILLATION

For convenience, cases of auricular fibrillation may be divided into three types: established, transient and paroxysmal.

1. Established: In this type the abnormal rhythm has been present for more than two weeks and has shown no tendency to alternate with sinus rhythm, and the presence of the irregularity is not dependent on a transient contributory factor.

2. Transient: This group includes the patients showing auricular fibrillation in association with some transient factor, in which the arrhythmia tends to disappear spontaneously after the contributory factor is no longer active.

3. Paroxysmal: Of this type are those patients who usually show sinus rhythm but who have periodic attacks of auricular fibrillation lasting from a few minutes to several days.

ESTABLISHED AURICULAR FIBRILLATION

Twenty patients selected from a total of eighty-eight with established auricular fibrillation were given quinidin. We would now exclude some of those treated, as others had been excluded, on account of evidence of serious degeneration of the ventricular myocardium. Inability to secure cooperation from some patients or their unwillingness to accept hospital admission prevented the inclusion of several more suitable patients. In no case was the drug given when well marked chronic passive congestion had recently been present, and still other patients who were deemed unsuited for this therapy were

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excluded because we doubted that their circulations would prove more efficient with sinus rhythm than with auricular fibrillation under digitalis control.

In the main, the therapeutic administration of quinidin to our established cases of auricular fibrillation covered a period of not more than one day. A test dose of 0.2 gm. or 0.4 gm. was given the first day in order to forecast if possible any unfavorable reaction to the drug, and during the day following, 2.4 gm. or less was administered in increments of 0.4 gm. It has been shown experimentally that quinidin is a cardiac poison, producing, in high concentration, ventricular tachycardia, difficulties in conduction, and finally ventricular fibrillation.[1] Moreover, various clinical observers have reported disturbing or serious results when this drug had been given in considerable dosage over a period of days. Sudden deaths have occurred, some due to embolism,[2] but others not clearly due to this cause.[3] These untoward results suggest caution.

The economic factor involved in quinidin therapy also contributed considerably to our decision to use this plan of treatment. Even after sinus rhythm is reestablished, practically all these patients relapse sooner or later into auricular fibrillation, and must be retreated with quinidin if it is desired to maintain regular rhythm. These repeated quinidin treatments necessitate hospital admissions since it is quite possible for unfavorable symptoms to arise in a second course (Case 1), even though the original experience has gone smoothly. However, we found that the difficulty or ease with which auricular fibrillation is interrupted by quinidin on the original treatment is apt to represent a fair forecast of the readiness with which regular rhythm may be again secured after relapse has occurred. And therefore it seems that if the original treatments depend for success on several days of high concentration of quinidin, then there will be a greater incidence of serious or disturbing reactions, and less likelihood of results that would prove of any lasting benefit to the patients.

All who were given quinidin had previously received sufficient digitalis to slow the ventricular rate below 90 per minute, the digitalis then being discontinued.

In those patients who could be successfully followed after the establishment of regular rhythm, quinidin was usually administered daily for a few weeks in doses of from 200 to 600 mg. in order to prevent an

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2. Wyckoff, J., and Ginsberg, M.: Boston M. & S. J. **190**:771-776 (May 8) 1924. Levine, S. A., and Wilmaers, Albert: Boston M. & S. J. **192**:388-392 (Feb. 26) 1925.

3. Levy, R. L.: Clinical Toxicology of Quinidin, J. A. M. A. **30**:1108-1113 (Sept. 30) 1922.

early recurrence of the irregularity. After this, on account of the fact that in many cases regular rhythm may be maintained unaided for long periods, the patients were permitted to do without the drug unless relapses occurred. Quinidin was retried in five cases and was successful in each instance.

Really satisfactory results, in which the patients were able to maintain a regular rhythm for long periods with apparently improved comfort and general health, except for brief lapses into auricular fibrillation, were obtained in four cases, or 20 per cent.

Regular rhythm was established in 55 per cent. of the entire group, and if the two patients are eliminated who responded unfavorably to the test dose the percentage in which regular rhythm was established was 61.

An attempt to analyze too minutely a short series of cases may lead one into error, but certain features of this short term plan of administering quinidin in twenty established cases of auricular fibrillation as evidenced in the accompanying table seem worthy of comment.

The cases were equally divided between the arteriosclerotic, the senile or the degenerative type, and those in which mitral disease was the main contributing factor, there being ten of each. In the mitral disease group regular rhythm was established in 70 per cent., while in the degenerative group only 40 per cent. became regular.

The average amount of drug necessary to bring about sinus rhythm in the mitral group was 1,142 mg., while in the degenerative group it was 1,450 mg.

The average duration of regular rhythm in the mitral group was ninety days, while in the degenerative group it was only twenty days.

Judging from the histories obtained from all the patients, the average length of time during which auricular fibrillation had been present in those who became regular after quinidin was five months; while in those who did not respond, the average duration had been nineteen months.

ELECTROCARDIOGRAPHIC CHANGES

Among the patients who regained normal rhythm the auricular waves were registered as coarse or moderately coarse in 75 per cent., but in only 33 per cent. of those in whom fibrillation persisted. Among the nine cases in which there was no response to quinidin were two cases of intraventricular block and one of right bundle branch block. After sinus rhythm had been established, two patients showed extrasystoles, one of auricular, and the other of nodal origin.

UNTOWARD SYMPTOMS

In two cases no more than a test dose was given on account of unfavorable response. Six patients were considerably nauseated; vomit-

Results of Treatment with Quinidin

	History of Duration of Fibrillation	Reaction to Test Dose	Number of Days Treated with Quinidin	Drug Discontinued Because of Unfavorable Symptoms	Total Amount of Drug Given, Gm.	Nausea	Vomiting	Diarrhea	Dyspnea	Increased Cyanosis	Rapid Ventricular Rate	Paroxysmal Auricular Tachycardia	Regular Rhythm	Duration of Regular Rhythm	Quinidin Retried After Relapse	Duration of Regular Rhythm on Retry	Improved General Health	Relative Cardiac Dulness, Cm. Left	Relative Cardiac Dulness, Cm. Right	Comment
													Mitral Disease Group							
1	2 mo.	None	1	0	2.0	0	0	0	0	0	0	0	+	5 mo.	3 times	17, 6, 9 mo.	++	16	× 3	Dyspnea; cyanosis; paroxysmal tachycardia and collapse following second treatment
2	Not known	None	1	0	0.4	0	0	0	+	+	+	+	+	4 hr.	0	0	0	10	× 3	
3	3 mo.	None	1	+	1.6	+	0	+	0	0	0	0	+	1 mo.	0	0	0	11	× 4	Patient unwilling to enter hospital for second treatment
4	3 mo.	None	1	0	0.4	0	0	0	0	0	0	0	+	1 mo.	+	Not known	?	11	× 4	Relapse was of paroxysmal type
5	3 mo.	None	1	0	0.4	0	0	0	0	0	0	0	+	6 days	0	0	0	12	× 4	Quinidin not retried on account of lack of cooperation of the patient
6	2 mo.	None	1	0	2.4	0	0	+	0	0	0	0	0	0	0	0	0	9½	× 3	
7	3 yr.	None	1	0	1.2	+	0	0	+	0	120	0	+	8 days	+	Not known	?	13	× 4	Patient went to South America
8	1 mo.	None	1	0	1.6	0	0	0	0	0	0	0	+	14 mo.	0	0	++	14	× 4	Still regular
9	Not known	None	1	+	1.2	+	0	+	0	0	0	0	0	0	0	0	0	13	× 4	
10	1 mo.	None	1	0	2.4	0	0	0	0	0	0	0	0	0	0	0	0	14½	× 4	Sudden death 29 hours after last dose of quinidin
													Degenerative Group							
11	1½ yr.	None	1	0	2.0	0	0	0	0	0	0	0	+	12 days	+	1 month	+	16½	× 5	
12	1½ yr.	None	1	+	1.2	+	0	0	+	+	0	0	0	0	0	0	0	14½	× 4	
13	1 mo.	None	1	0	1.6	0	0	0	0	0	0	0	+	2 wk.	0	0	0	13½	× 3	Did not return after relapse
14	4 yr.	None	1	+	0.4	0	0	0	0	0	0	0	0	0	0	0	0	13	× 4	Drug discontinued on account apprehension and depression
15	2 yr.	None	1	0	2.4	0	0	0	0	0	0	0	0	0	0	0	0	14½	× 3	
16	3 mo.	Nausea	0	+	0.4	+	+	0	+	+	120	0	0	0	0	0	0	10	× 3	
17	Not known	Nausea	0	+	0.4	+	0	0	+	+	120	0	0	0	0	0	0	14	× 4	
18	2 yr.	None	2	0	3.8	0	0	0	0	0	0	0	0	0	0	0	0	13	× 4	
19	Not known	None	1	0	1.0	0	0	0	0	0	0	0	+	3 days	+	Not known	?	13	× 4	Did not return after second treatment
20	Not known	None	1	0	1.2	0	0	0	0	0	0	0	+	6 wk.	0	0	+	13	× 4	Lived at a distance, could not be followed; relapses of paroxysmal type

ing occurred in one; diarrhea in three; dyspnea in five; increased cyanosis in five; dizziness in two; the ventricular rate rose above 120 in four; paroxysmal auricular tachycardia occurred in two, and collapse in one. In the following case, death occurred twenty-nine hours after quinidin had been discontinued, the heart having failed to become regular. This patient had mitral disease and a large heart.

REPORT OF CASE

CASE 1.—A woman, aged 22, was admitted to the hospital, Feb. 2, 1923, complaining of rapid heart action. She had had diphtheria at eight years and rheumatic fever at the age of twenty. Tonsillectomy had been done three years previous to the rheumatic attack.

The onset of the present illness followed rheumatic fever two years before her admission. There was then some palpitation for a month or so with subsequent improvement. Rapid and irregular heart action had been present for the last month.

There was slight cyanosis of the lips but no dyspnea or edema. The tonsillar tissue had recurred on the left, and the thyroid gland showed some diffuse enlargement. The cardiac dulness was considerably enlarged to the right and the left, the relative dulness measuring 14.5 cm. to the left of the midsternal line in the fifth interspace and 4 cm. to the right in the fourth. The heart's action was completely irregular. The apex rate was 140 per minute and the radial count was 110. There was a harsh, blowing systolic murmur at the apex. The second pulmonic sound was accented. The liver edge was at the costal margin; there were no râles at the bases of the lungs, and there was no edema of the lower extremities.

The patient was given 4 drams (15 c.c.) of tincture of digitalis over a period of three days, the pulse deficit disappearing and the apex rate slowing down to 70 per minute, though still completely irregular.

On the fourth day, a test dose of 0.4 gm. of quinidin was given without any reaction. The day following 2 gm. was administered in increments of 0.4 gm. The cardiac action continued irregular and on the next day was 90 per minute. No disturbing gastro-intestinal symptoms were noted. At 9:15 p. m., twenty-nine hours after the last dose of quinidin, the patient remarked to the nurse who was in the room how well she felt, when suddenly her facial muscles began to twitch, she rapidly became cyanotic and dyspneic, and died in a few moments. On the last observation, shortly before the patient's sudden death, the pulse had been completely irregular. A necropsy could not be secured.

It does not seem possible to draw a clear conclusion as to the cause of death in this case. It may be said that it should not necessarily be charged to quinidin. Lewis [4] has shown that quinidin is very quickly destroyed in the body, and in twenty-four hours is apt to be largely eliminated. At the end of this period it is doubtful whether regular rhythm and resulting cerebral embolism would ensue from quinidin if it has not occurred during the higher concentration of the drug administered the preceding day. The death may have been due to cerebral embolism unrelated to quinidin administration. However, it seems difficult to escape the probability that there was some relation to the drug therapy.

4. Lewis, T.: Am. J. M. Sc. **163**:781 (June) 1922.

TRANSIENT AURICULAR FIBRILLATION

Instances of transient auricular fibrillation, according to our definition, have been so frequent in our experience that we are not inclined to include the detailed case reports. However, the principal contributing factors, in the order of their frequency, have been:

1. Thyroidectomy.
2. Toxic adenoma of the thyroid.
3. Major surgical operations, such as cholecystectomy.
4. Acute lobar pneumonia.
5. Certain miscellaneous cases, which have included exhaustion from extreme overexertion, acute alcoholism, starvation and dehydration in association with acute cholecystitis.

As is well known, auricular fibrillation develops frequently after thyroidectomy. It commonly occurs the first or second day after operation and constitutes a disturbing but not necessarily serious complication. Its occurrence is an indication for digitalization of the patient, a procedure that is generally sufficient to increase his comfort, the irregularity of the pulse disappearing spontaneously in twenty-four or forty-eight hours. In cases in which the arrhythmia persists on into convalescence, it is, as a rule, easy to produce a reversion of sinus rhythm with quinidin.

Toxic adenoma of the thyroid, occurring as it does largely in people over 50 years of age, is also a potent factor in the production of auricular fibrillation. The classification of the arrhythmia in these instances as transient, even when the irregularity has been present for long periods—months or years—may be questioned. It is true that the arrhythmia does persist after thyroidectomy in some of these patients, but, at least before operation, the auricular fibrillation should always be thought of as potentially transient. In the majority of our patients with hyperthyroidism and auricular fibrillation who have had thyroidectomy, the arrhythmia has disappeared spontaneously during convalescence, without any need for quinidin therapy. Our experience in this regard is in accord with that of Dunhill, Fraser and Stott,[5] who likewise noted a spontaneous resumption of normal rhythm in thyrotoxic auricular fibrillation after removal of the thyroid in a considerable proportion of the cases.

It would seem that in transient auricular fibrillation, quinidin, although of occasional value, is seldom indicated, since the tendency is for spontaneous resumption of normal rhythm after the elimination of the transient factor.

5. Dunhill, T. P.; Fraser, F. R., and Stott, A. W.: Quart J. Med. **17**: 326-338 (July) 1924.

PAROXYSMAL AURICULAR FIBRILLATION

Instances of paroxysmal auricular fibrillation, or flutter, associated with hyperthyroidism are excluded from this report, since they are best treated by thyroidectomy.

CASE 2.—A university professor, aged 68, for six years had been subject to attacks of auricular fibrillation at intervals of a month or more. In the last year the attacks had been occurring more frequently, at intervals of every two weeks. The physical examination showed no remarkable cardiac findings. The blood pressure was 144 systolic and 80 diastolic. There was no marked evidence of arteriosclerosis and no obvious cardiac hypertrophy. An electrocardiogram was made during the period of regular rhythm and showed no abnormality.

This patient began taking quinidin sulphate, 200 mg. daily, and continued this for six weeks. During that time he did not feel that there was any decrease in the frequency of his attacks or any shortening of the attacks that did occur.

CASE 3.—An Arabian woman, aged 50, who was admitted to the hospital, March 7, 1925, stated that for five years she had been subject to spells of palpitation, or heart fluttering. During the last month, these attacks had been especially frequent, coming on every four or five days and being aggravated by exertion.

The patient was a very obese woman. The cardiac rhythm was completely irregular; the rate was 150. The pulse rate was 80. During the examination, the rhythm suddenly became regular and the patient announced that she felt better again. An electrocardiogram made during the period of regular rhythm showed no abnormality.

The patient was placed on quinidin sulphate, 400 mg. twice a day, and this was in a short time reduced to 200 mg. twice daily. Under this regimen the frequency of the attacks was diminished from every few days to every two weeks, and the length of the attacks diminished from an average of from four to ten hours to an average of one-half hour.

CASE 3.—A man, aged 50, who had been followed since April, 1920, had been subject to periodic attacks of palpitation since February, 1920. The personal history was unimportant.

The blood pressure was 145 systolic and 80 diastolic. The relative cardiac dulness extended from 10.75 cm. to the left in the fifth interspace to 3 cm. to the right in the fourth interspace. The heart action was unusually regular. There was a soft systolic murmur at the apex and also in the aortic area. An electrocardiogram showed evidence of incomplete bundle branch block.

He had been observed repeatedly during paroxysms of auricular fibrillation. These had occurred at irregular intervals, seldom not more frequently than once a month, and often he had freedom from paroxysms for six months. The average duration of the attacks had been from six to ten hours. During an attack the administration of 400 mg. of quinidin was tried but it seemed to increase the palpitation and did not serve to terminate the attack in a shorter time than usual. In the majority of the paroxysms, he had been given tincture of digitalis, 1½ drams (5.6 c.c.), and this had served to diminish the palpitation within an hour or two and seemed to shorten the attacks.

CASE 4.—A woman teacher, aged 52, was admitted to the hospital, Nov. 10, 1923, complaining of heart trouble. There was a previous history of acute rheumatic fever, tonsillitis and scarlet fever as a child.

The patient had known that she had some heart trouble for the last ten years. One year before admission, she developed attacks of rapid and irregular heart action, which occurred with great frequency and sometimes lasted for twenty-four hours. These attacks had been brought on by eating or by any exertion.

On examination she proved to be considerably undernourished. The cardiac apex impulse was felt in the sixth interspace, 7 cm. to the left of the midsternal line. The cardiac dulness extended 7 cm. to the left in the fifth interspace and 4 cm. to the right in the fourth interspace. The first sound at the apex was very

snapping. There was a blowing systolic murmur at the apex and also a faint presystolic rumbling murmur.

During the periods of regular rhythm, the electrocardiogram showed some T wave flattening and auricular extrasystoles. The P waves in Lead II were somewhat enlarged. Auricular fibrillation developed frequently after the eating of a meal, and was observed to be brought on after exercise.

This patient was given quinidin sulphate, 200 mg. twice daily. Two days after this was begun, the heart was again observed to be irregular and the drug was increased to 600 mg. daily, in increments of 200 mg., and this was sufficient to maintain normal rhythm. This patient has continued to take quinidin daily with the most remarkable improvement in her comfort and health. Although she had previously been completely disabled from teaching, she returned to her work after suitable convalescence and has continued at it ever since. In her own words, she has been on "full time work with a very heavy schedule." There have been only two very brief attacks of palpitation during a term.

It is interesting that over a short period of time this patient was given quinin, through a pharmacist's mistake, and suffered a considerable increase in the frequency of the attacks. This was corrected as soon as the error was detected and quinidin was again brought into use.

No suggestive evidence of thyroid disease was observed in any of these patients.

In paroxysmal auricular fibrillation not due to hyperthyroidism, quinidin is occasionally useful in relieving the patient of disagreeable or annoying palpitation, and this may be the only favorable effect. But in some cases the disability that has resulted from very frequent paroxysms may be quite severe, as in Case 4, so that the drug may at times succeed in restoring to useful occupation patients who would otherwise have greatly diminished working efficiency.

It would seem that the prophylactic use of quinidin is not necessary unless the paroxysms occur more frequently than every two weeks. We are not much inclined to use the drug in the treatment of individual paroxysms unless they tend to be prolonged and then only after preliminary digitalization.

Our most satisfactory results have been secured in patients who had had frequent attacks. In these, the prophylactic use of from 0.2 to 0.8 gm. of quinidin daily increased the period of freedom from attacks and considerably shortened the length of the paroxysms that did occur. The absence of this effect in Case 2 was probably due to insufficient dosage.

SUMMARY AND CONCLUSIONS

Established Auricular Fibrillation.—1. We have had 61 per cent. success in the production of normal rhythm from auricular fibrillation by a one day plan of quinidin administration. This compares favorably with the results obtained in a large number of cases already reported (56 per cent.).[6]

6. Burwell, C. S., and Dieuaide, F. R.: Clinical Experience with Quinidin, Arch. Int. Med. **31**:518-525 (April) 1923.

2. The patient with mitral disease who has had the arrhythmia for a relatively short time is most likely to revert to normal rhythm after quinidin, and in such patients the resulting regular rhythm will likely persist longer than in the arteriosclerotic or degenerative type.

3. Patients of the arteriosclerotic or degenerative type who have been irregular for long periods are often more difficult to restore to normal rhythm by quinidin; and such rhythm if produced is apt to be of relatively short duration.

4. Patients with coarse electrocardiographic auricular waves are more easily rendered regular by quinidin than those who show fine auricular waves.

5. One death occurred in association with quinidin therapy, in our patients.

6. In the mitral disease group, quinidin should be used only by those patients without marked cardiac hypertrophy and dilatation who have had the irregularity for but a short time and who have not had congestive cardiac failure.

7. In the degenerative group, we are inclined to believe that the use of quinidin is not justified.

8. In no case of established auricular fibrillation should this drug be used unless it is apparent that close cooperation is going to be possible between the patient and the physician, and unless hospital admissions and readmissions can be readily arranged if thought necessary.

Transient Auricular Fibrillation.—Quinidin was found indicated only when the arrhythmia persisted after the disappearance of the transient causative factor. In cases associated with hyperthyroidism, quinidin apparently should not be used until six weeks after thyroidectomy.

Paroxysmal Auricular Fibrillation.—1. In those cases not due to hyperthyroidism in which the paroxysms were frequent, quinidin administered prophylactically, in doses of from 0.2 to 0.4 or 0.8 gm. daily, was found to be of the greatest value in lengthening the interval between the attacks and in shortening the paroxysms that did occur.

2. In the treatment of individual paroxysms, quinidin need be used only in rare instances when the paroxysm tends to be prolonged.

3. Quinidin was not found necessary in daily dosage unless the paroxysms occurred more often than once in two weeks.

The chief value of quinidin was found in the treatment of frequent paroxysmal auricular fibrillation when not associated with hyperthyroidism. It has many dangers and an extremely limited field of usefulness in the established cases, and real benefit of any considerable duration is derived in only a small proportion of these.

THE VALUE OF THE ICTERUS INDEX IN DIFFERENTIATING ANEMIA*

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Everyone realizes the pitfalls and difficulties that all except the most highly trained hematologists encounter in the differentiation of the anemias. Mistakes in diagnosis are not uncommon; especially is this true when the busy clinician mistakes a severe secondary anemia, such as may occur in connection with carcinoma, for a case of pernicious anemia.[1] In just such types of cases anything that may aid in making a correct diagnosis simply and rapidly is worthy of consideration. Our aim, therefore, was to determine what clinical value, if any, the icterus index might have in this direction.

We have interested ourselves primarily with a clinical fact, namely, the intensity of the yellow color of the blood serum, and in this study have not concerned ourselves particularly with the numerous considerations as to the possible sources of this color.

As this was to be a clinical test, the simplest and most rapid means of determining the icterus index was desired. Hence, we made use of a modification of Meulengracht's bilirubin colorimeter.[2] For his standard, he uses 0.05 parts of potassium dichromate in 500 c.c. of distilled water, with 2 drops of sulphuric acid; this gives a depth of color corresponding to an icterus index of 1. He then determined the bilirubin content of the blood as shown by the amount of physiologic sodium chlorid solution that has to be added to the plasma to bring the tint to correspond with the standard. By our modification, using the same standard solution for an index of 1 and having a series of permanent standards of greater intensity of color, the color comparison is made directly; this does away with the necessity of diluting the specimen in a manner similar to that done by the Dunning apparatus of phenolsulphonephthalein estimation in the urine.

It is understood that the method of Hymans-Van den Bergh[3] for the quantitative estimation of bilirubin in the serum is probably slightly more accurate, but we believe that this added accuracy is clinically

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1. St. George, A. V.: Proc. New York Path. Soc. **22**:6-8, 1922.
2. Meulengracht, E.: Deutsch. Arch. f. klin. Med. **137**:38 (Aug.) 1921.
3. Van den Bergh, A. A.; Hymans, and Snapper: Deutsch. Arch. f. klin. Med. **110**:540, 1913.

unnecessary and that, for ordinary clinical purposes, the test is needlessly complex of performance so far as the differentiation of the anemias is concerned.

In order to obtain our own figures for the normal range of the icterus index, the determination was made on the plasma of 120 consecutive nonprofessional blood donors, ranging in age from 18 to 47 years. These donors were relatives or friends of patients in the wards of Bellevue Hospital who were in need of transfusions, and the donors, as far as they themselves knew and to all outward appearances, formed a group of average healthy adults.

From Table 1 and the accompanying chart it will be noted, first, that the average index was 4.41, and, second, that 90.8 per cent. of the control cases had an index between 3.5 and 5.5, inclusive. Van den Bergh [2]

TABLE 1.—*Icterus Index Determinations in One Hundred and Twenty Controls; Average Index, 4.41*

Icterus Index	Number of Cases
2.0	1
2.5	0
3.0	4
3.5	15
4.0	29
4.5	38
5.0	19
5.5	8
6.0	3
6.5	0
7.0	2
7.5	0
8.0	0
8.5	0
9.0	1

obtained similar figures. This suggests a rather well defined range of normal variation, and when a determination is obtained that is either above or below this range, a clinical cause therefore should be sought. It is altogether probable, had we been fortunate enough to be able to make a thorough examination of the donors with indexes outside this range, that in the majority of cases the cause of the variation from the so-called normal could have been determined. Thus, we found that the lone index of 9 belonged to a rather obese Italian woman, aged 44, giving a typical history of chronic cholecystitis.

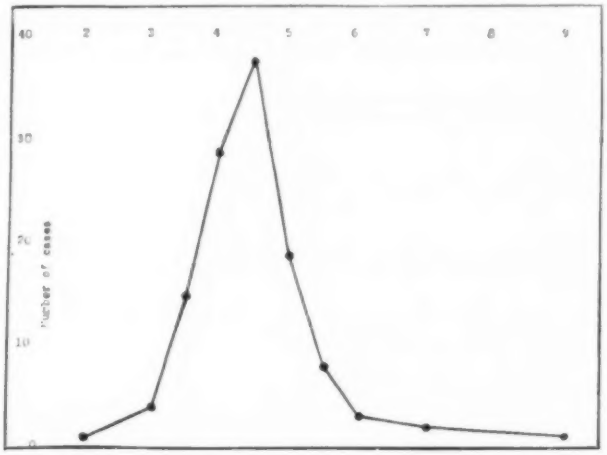
Our own series of clinical cases includes twenty-three cases of pernicious anemia, twenty-three cases of carcinoma with anemia, fourteen examples of secondary anemia and eighteen conditions other than pernicious anemia giving a high index.

Let us consider, first, the cases of pernicious anemia (Table 2): This series was selected for the reason that it includes only those in which the final diagnosis was confirmed beyond a reasonable doubt. We were fortunate enough subsequently to confirm two of these diagnoses by postmortem examination. The results are very consistent, the icterus

index in every case being well above the range of normal variation. In uncomplicated cases the range varied from 9 to 12.5, with an average index of 9.7.

The fact that we found an increased index in pernicious anemia is in accord with the work of other investigators. Andrewes [4] presents a series of such cases, all of which gave a Van den Bergh indirect test above the normal range. Hirschfeld [5] and Lepehne [6] draw similar conclusions.

By complicated cases we mean those in which some factor other than hemolytic anemia is working to give a high icterus index, as in Cases 30, 34 and 36, in which the cardiac failure produced more or less



Range of icterus index in 120 control cases; average icterus index, 4.41; 90.8 per cent. of all between index of 3.5 and 5.5.

marked hepatic obstruction. If, perchance, the question arises as to whether the high index in a particular case is due to hemolytic anemia or to hepatic obstruction, then Van den Bergh's direct test may be performed. (The serum from cases of obstructive jaundice gives a direct reaction, and normal serums or those from hemolytic anemias give the indirect reaction only.)

No constant relationship was noted between the height of the index and the severity of the anemia. This seems to be dependent, rather, on the phase of the cycle during which blood is taken from the patient suffering from pernicious anemia. Thus, during a bone marrow crisis

4. Andrewes, C. H.: Quart. J. Med. **18**:69, 1924.
5. Hirschfeld: Ztschr. f. klin. Med. **87**:1, 1919.
6. Lepehne, G.: Deutsch. Arch. f. klin. Med. **135**:79 (Jan.) 1921.

TABLE 2.—*Icterus Index in Pernicious Anemia*

Case Number	First Diagnosis	Final Diagnosis	Kind of Diagnosis	Icterus Index	Jaundice	Sex*	Age	Hemorrhage	Red Blood Cells	Hemoglobin, per Cent.	Remarks
1	Pernicious anemia	Pernicious anemia	Clinical	10.0	0	♂	32	0	1,600,000	45	Blood picture typical
2	Acute encephalitis?	Pernicious anemia	Clinical	8.5	0	♀	..	0	3,300,000	56	
3	Pernicious anemia	Pernicious anemia	Clinical	9.0	Italian 0	♀	..	0	2,500,000-1,296,000	45-40	
4	Pernicious anemia	Pernicious anemia	Clinical	9.0	Lemon	♂	..	0	1,270,000	50	
5	Pernicious anemia	Pernicious anemia		9.0	0	♂	..	0	2,800,000-3,600,000	60	
6	Pernicious anemia	Pernicious anemia	Clinical	9.0	0	♂	55	0	1,500,000	40	
7	Pernicious anemia	Pernicious anemia	Clinical	11.5	±	♀	47	0	416,000	20	
8	Pernicious anemia	Pernicious anemia	Necropsy	8+	0	♀	..	0	1,800,000-2,600,000	30-80 D	
30	Pernicious anemia	Pernicious anemia	Clinical	45.0	++	♂	..	0	800,000	29	Cardiac decompensation +
31	Pernicious anemia	Pernicious anemia	Clinical	12.5	Lemon	♂	67	0	1,440,000	32	
32	Pernicious anemia	Pernicious anemia	Clinical	8.5	Lemon	♀	60	0	1,350,000	40	
33	?	Pernicious anemia	Clinical	9.5	Pallor	♀	40	0	1,808,000	45	Liver and spleen +
34	Hemolytic jaundice	Pernicious anemia	Clinical	16.0	+	♀	56	0	900,000	30	Cardiac decompensation +
35	Secondary anemia	Pernicious anemia	Necropsy	11.5	Lemon	♀	25	0	720,000	25	
36	Pernicious anemia	Pernicious anemia	Clinical	14.0	Lemon	♂	65	0	900,000	35	Chronic cardiovalvular disease
37	Pernicious anemia	Pernicious anemia	Clinical	9.0	Lemon	♀	79	0	770,000	30	
38	Pernicious anemia	Pernicious anemia	Clinical	11.0	±	♀	49	0	1,790,000	60	
39	Pernicious anemia	Pernicious anemia	Clinical	8.0	0	♀	39	0	1,130,000	25	
40	Carcinoma (?) or pernicious anemia	Pernicious anemia	Clinical	12.5	±	♀	54	0	3,000,000	60	
71	Pernicious anemia	Pernicious anemia	Clinical	11.5	Lemon	♂	56	0	1,490,000	45	
72	Pernicious Anemia	Pernicious anemia	Clinical	8.5	±	♀	40	0	2,000,000	50	Splenectomy 1 year
73	Carcinoma of stomach	Pernicious anemia	Clinical	9.0	Lemon	♂	48	0	1,000,000	37	Negative gastro-intestinal roentgen-ray examination
74	Pernicious anemia	Pernicious anemia	Clinical	8.5	Pallor	♀	58	Slight	2,100,000	60	Ulcerative colitis

* In this and the following tables ♂ indicates male, ♀ female.

or even before the crisis, the index mounts rapidly day by day, and, similarly, before obvious clinical improvement the index gradually falls toward the normal. This suggests a clinical means of determining when it would be advisable to transfuse a pernicious anemia patient, as well as when the patient might improve spontaneously.

Table 3 consists of twenty-three cases of carcinoma with anemia. The results here also were uniformly consistent in that the icterus index was invariably at or below the normal range. Over half of these cases proved to be carcinoma on histologic examination of the new growth. In the other cases, the growth was so far advanced as to be declared inoperable.

It is well known that anemia due to an obscure new growth and pernicious anemia not infrequently present confusingly similar blood pictures to the average laboratory man, to say nothing of the busy practitioner. Similarly, as shown by Warfield,[7] chronic infections often give a picture that on cursory examination is diagnosed as pernicious anemia. Cases 93 to 97, inclusive, are examples of this condition. In Case 97, the diagnosis made at necropsy differed from that made during life, which was pernicious anemia. The patient was a man, aged 45, who had been ill for about six weeks. He was jaundiced; the liver and spleen were enlarged; the red cells totaled 610,000 and hemoglobin 15 per cent., with all forms of abnormal red cells; the Wassermann reaction was ++++ repeatedly. At the time we saw the patient, the following note was made, on the basis of the icterus index, which was 46.

> The height of this index cannot be accounted for by a simple case of pernicious anemia nor by uncomplicated Banti's disease. However, either of the above, plus cardiac decompensation, producing obstruction (passive congestion) in the liver, could give such an index. Again, if the anemia is the sole cause, there would have to be a tremendous amount of active blood destruction, as occasionally occurs in acute arsphenamin poisoning.

In the light of our present knowledge, we could also have considered the possibility of chronic sepsis. The necropsy note by Dr. Douglas Symmers contains the following statement: "At autopsy, the whole picture appeared to be easily explained on the basis of chronic sepsis with hemolytic jaundice."

This brings forth the possibility of the facile use of the icterus index as an aid in the differential diagnosis, not only of cases of hemolytic anemia from secondary anemia, but more especially of cases of pernicious anemia from anemia due to carcinoma. Thus, if we glance at Tables 2 and 3, we note that, irrespective of the degree of the anemia, the icterus index in cases of pernicious anemia was uniformly above the

7. Warfield, L. M.: New York State J. Med. **25**:147 (Feb. 6) 1925.

TABLE 3.—*Icterus Index in Carcinoma with Anemia*

Case Number	First Diagnosis	Final Diagnosis	Kind of Diagnosis	Icterus Index	Jaundice	Sex	Age	Hemorrhage	Red Blood Cells	Hemoglobin, per Cent.	Remarks
9	Carcinoma of stomach.....	Carcinoma of stomach.....	Operation	1.5	0	♀	42	0	3,820,000	60	Pathologic specimen
10	Carcinoma of breast.....	Carcinoma of breast.....	Clinical	3.0	0	♀	..	0	4,005,000	55	Inoperable
11	Carcinoma of breast.....	Carcinoma of breast.....	Clinical	1.6	0	♀	45	Slight + breast	3,840,000	50	Inoperable
12	Carcinoma of prostate, metastases to liver	Carcinoma of prostate, metastases to liver	Clinical	2.5	0	♂	71	0	3,200,000	65	Proctoscopy
13	Carcinoma of rectum.....	Carcinoma of rectum.....	Clinical	2.0	0	♂	46	+ + + +	3,100,000	70	Proctoscopy
14	Carcinoma of rectum.....	Carcinoma of rectum.....	Clinical	3.0	0	♀	44	0	3,500,000	40	Proctoscopy
15	Carcinoma of stomach.....	Carcinoma of stomach.....	Clinical	2.5	0	♀	63	0	.....	..	Inoperable
16	Carcinoma of penis, chest.....	Carcinoma of penis, chest....	Operation	3.0	0	♂	51	0	3,450,000	60	Pathologic specimen
17	Carcinoma of stomach.....	Carcinoma of stomach.....	Clinical	2.0	0	♀	61	+ +	2,000,000	..	Roentgen-ray examination
18	Carcinoma of stomach.....	Carcinoma of stomach.....	Operation	3.0	0	♂	..	0	4,500,000	90	Pathologic specimen
19	Carcinoma of sigmoid.....	Carcinoma of sigmoid.....	Necropsy	11.0	0	♀	..	0	2,240,000	..	Pneumonia
20	Carcinoma of ulcer of stomach	Carcinoma of stomach.....	Operation	2.5	0	♀	47	0	4,400,000	65	Preoperative diagnosis: by icterus index, carcinoma; clinically inoperable
21	Carcinoma of stomach.....	Carcinoma of stomach.....	Clinical	1.5	0	♀	61	....	2,000,000	33	Roentgen-ray examination
22	Carcinoma of sigmoid.....	Carcinoma of sigmoid.....	Operation	2.5	0	♂	55	0	3,100,000	40	Inoperable by exploration
23	Carcinoma of sigmoid.....	Carcinoma of sigmoid.....	Operation	2.5	0	♂	60	Slight repeated	3,980,000	65	Pathologic specimen
24	Carcinoma of sigmoid.....	Carcinoma of sigmoid.....	Operation	2.0	0	♀	55	Repeated	3,740,000	70	Inoperable—colostomy
25	Carcinoma of rectum.....	Carcinoma of rectum.....	Operation and biopsy	2.0	0	♂	66	Repeated	3,770,000	65	Pathologic specimen—inoperable
26	Carcinoma of larynx.....	Carcinoma of larynx.....	Operation	2.0	0	♂	53	0	3,670,000	65	Operation; died
27	Carcinoma of rectum.....	Carcinoma of rectum.....	Biopsy	1.5	0	♀	65	+ +	2,460,000	40	Biopsy
28	Carcinoma of esophagus.....	Carcinoma of esophagus.....	Operation	2.0	0	♂	54	0	3,110,000	40	Operation; pathologic specimen
29	Carcinoma of tongue.....	Carcinoma of tongue.....	Operation	3.0	0	♂	59	0	4,100,000	75	Biopsy
66	Carcinoma of breast.....	Carcinoma of breast.....	Clinical	2.0	0	♀	50	0	2,740,000	40	Inoperable
67	Carcinoma of stomach.....	Carcinoma of stomach.....	Operation	1.5	0	♂	54	0	.....		Pathologic specimen; secondary anemia

TABLE 4.—*Various Conditions Exhibiting a Low Icterus Index*

Case Number	First Diagnosis	Final Diagnosis	Kind of Diagnosis	Icterus Index	Jaundice	Sex	Age	Hemorrhage	Red Blood Cells	Hemoglobin, per Cent.	Remarks
41	Pernicious anemia	Hemophilia	Clinical	1.5	Lemon	♂	23	+++	1,350,000	15	
42	Pernicious anemia? Leukemia?	Leukemia	Clinical	5.0	0	♂	60	0	1,940,000	35	
43	Pernicious anemia?	Hookworm	Organisms demonstrated	3.0	Pallor	♂	..	+	2,410,000	55-22	
44	Uncinariasis	Hookworm	Organisms demonstrated	2.0	0	♂	32	0	2,900,000	65	
45	Pernicious anemia?	Splenic anemia	Clinical	3.5	±	♂	45	0	2,002,000	40	Enlargement of liver and spleen; Wassermann reaction ++++
46	Sarcoma	Sarcoma thigh	Necropsy	1.8	Pallor	♂	..	0	4,070,000	65	
47	Fibromyomas	Fibromyomas	Operation	1.0	Pallor	♀	47	Repeated ++++	2,400,000	40	Pathologic specimen
48	Ulcerative colitis	Ulcerative colitis	Clinical	1.5	Pallor	♂	39	Repeated ++++	2,765,000	30	Proctoscopy and stool examination; alcoholic 14 years
49	Lymphosarcoma of neck	Lymphosarcoma of neck	Necropsy	2.0	Pallor	♂	54	0	4,400,000	60	
50	Pernicious anemia?	Specific anemia	Clinical	2.5	Pallor	♂	40	0	1,880,000	25	Wassermann repeatedly +; blood picture not like pernicious anemia
51	Hodgkin's disease	Hodgkin's disease	Operation	2.0	Pallor	♂	38	0	2,360,000	40	Pathologic specimen
52	Pernicious anemia	Bleeding fibroid	Operation	3.0	0	♀	..	Repeated +++	1,800,000	40	Well at present (5/14/25)
53	Cholelithiasis?	Bleeding gastric ulcer	Operation	3.0	0	♀	40	++	1,740,000	35	Blood returned to normal after operation
23	Carcinoma of sigmoid	Carcinoma sigmoid	Operation	2.5	0	♂	60	Repeated +	3,980,000	60	Pathologic specimen

TABLE 5.—*Various Conditions Exhibiting a High Icterus Index*

Case Number	First Diagnosis	Final Diagnosis	Kind of Diagnosis	Icterus Index	Jaundice	Sex	Age	Hemorrhage	Red Blood Cells	Hemoglobin, per Cent.	Remarks
80	Familial jaundice	Familial jaundice	Clinical	14.0	±	♀	5	0	2,976,000 to 3,520,000	32.5 to 50	Wassermann negative
81	Carcinoma or common duct obstruction	Common duct obstruction	Operation	104.0	++++	♂	..	0	.....	..	Mild secondary anemia
82	Carcinoma of pancreas	Chronic cholecystitis and cholelithiasis	Operation	73.0	+++	♂	35	0	3,232,000	35	
83	Weil's disease	Weil's disease	Clinical	96.0	++++	♂	..	0	.....	..	Secondary anemia
84	Polycythemia vera	Polycythemia vera	Clinical	7.0	0	♀	55?	0	6,300,000	85	
85	Cholecystitis	Chronic cholecystitis	Operation	16.0	+	♀	45?	0	4,904,000	65	Pathologic report
86	Pernicious anemia	Arsphenamin poisoning	Clinical	8.0	0	♂	..	0	Patient had anemia of 3,000,000, followed by drop to 1,000,000 (with two doses of arsphenamin)		
87	Aplastic anemia	Benzene poisoning	Clinical	8.5	Pallor	♂	30?	...	.....	23 to 57	
88	Duodenal ulcer	Duodenal ulcer	Operation	8.0	Pallor	♂	49	0	4,800,000	75	Pathologic specimen
89	Typhoid fever	Typhoid fever	Clinical	9.5	0	♀	30?	0	4,490,000	80	Positive Widal and blood culture
90	Acute cholelithiasis	Acute cholelithiasis	Operation	10.5	0	♂	47?	0	5,600,000	60	During attack
91	Acute cholecystitis	Chronic cholecystitis, etc.	Operation	39.0	++	♀	46?	0	3,440,000	55	Pathologic specimen
92	Diabetes	Diabetes	Clinical	9.0	0	♀	46	+ sl.	4,240,000	70	
93	Acute appendicitis	Acute appendicitis and general peritonitis	Operation	9.5	±	♂	..	0	4,820,000	85	Associated pericholangitis; Van den Bergh slightly +; bilirubin 50 mg. per 100 c.c.
94	Carcinoma of head of pancreas	Cirrhosis of liver and chronic pancreatitis	Operation	96.0	++++	♂	63	0	4,560,000	85	Wassermann reaction 4+
95	Acute gangrenous appendicitis	General sepsis	Operation	8.0	0	♀	19	0	4,740,000	75	Pylephlebitis; acute peritonitis; died
96	Acute appendicitis with abscess	Acute appendicitis with abscess and sepsis	Operation	29.0	+	♂	32	0	4,280,000	80	Discharged improved
97	Pernicious anemia	Chronic sepsis and hemolytic jaundice	Necropsy	46.0	++	♂	45	0	610,000	15	Wassermann reaction 3+

normal range, and in cases of carcinoma it was below normal. The interesting point is that not only both series have indexes differing from normal and from one another, but that each range is on the opposite side of normal, making differentiation by means of the icterus index relatively simple.

The fact that hemolytic anemia can be readily differentiated from anemia due to other causes, by means of the icterus index, has been pointed out before by Lepehne [6] and by Andrewes.[4]

Table 4 presents examples of various conditions in which there was secondary anemia with a low index. Uncomplicated cases of secondary anemia are thus seen to give a uniformly low normal or below normal index. This table also is of special interest for the reason that the cases contained therein were at first wrongly diagnosed clinically as pernicious anemia, and finally turned out to be secondary anemias of one sort or another (Cases 41, 43, 45 and 50).

It is not to be supposed that a diagnosis of pernicious anemia is to be made merely because the patient presents a high index with more or less marked anemia. Thus, Table 5 gives numerous examples of conditions other than pernicious anemia which may give a high index. Many of these conditions can be differentiated with ease clinically and by the history, etc., or with the additional information supplied by Van den Bergh's test.

Likewise, complicating factors must always be borne in mind in the interpretation of any index reading. For example, what would ordinarily be considered a normal or above normal index might be lowered because of hemorrhage, or, on the other hand, the index in a particular case might be raised by such factors as coexisting pneumonia, cardiac insufficiency, or even a slight chronic cholecystitis. Case 19, Table 3, illustrates the point in question. Here we have a case of carcinoma of the sigmoid (proved at necropsy) with severe anemia, showing no metastases in the liver or in the biliary system, but nevertheless giving a high icterus index. The answer lies in the terminal pneumonia which, through hypostatic congestion, caused biliary stasis and gave the raised icterus index. Here, as with almost everything else in medicine, the personal equation must be brought to bear.

SUMMARY

1. The icterus index, rationally interpreted and performed by a simple method, is a distinct clinical aid in the differentiation of the anemias.

(*a*) Uncomplicated cases of hemolytic anemia have an index above the normal range.

(*b*) Uncomplicated cases of secondary anemia have a low index, generally below the normal range.

2. Uncomplicated cases of pernicious anemia and the severe anemia simulating it in cases of carcinoma may be readily differentiated by the icterus index, in that the former have an index consistently above the normal (8 to 12.5) and the latter below the normal range.

3. (*a*) The most common factors to be borne in mind which may raise the icterus index above the usual for the primary disease in the subject in question are coexisting pneumonia, cardiac insufficiency and chronic sepsis (especially in the biliary system).

(*b*) The factor that may lower the icterus index below the usual for the disease in question is hemorrhage, especially a constant, slow (oozing) bleeding, such as occurs in gastro-intestinal new growths.

4. The normal range of the icterus index, as determined by the modified Meulengracht method, is from 3.5 to 5.5, with an average of 4.41, for 120 cases.

5. It is suggested that the icterus index may be used to follow the course of pernicious anemia and determine, before the blood count falls, when it is advisable to give a transfusion, as well as when the patient is likely to improve spontaneously.

THE CONCENTRATION OF THE BLOOD AND OF THE URINE IN DIABETIC TOXEMIA*

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The marked concentration of the blood that is found in the severe diabetic state has been pointed out in a previous article.[1] Here we wish to show the striking changes in the concentration of the urine that are associated with this condition and to indicate the rapid alterations that accompany successful therapy.

Urinary nitrogen was determined by the usual Kjeldahl method; sugar by a modification of Benedict and Osterberg's micro method [2] in the earlier studies and later by the Shaffer and Hartmann method.[3] Urinary phosphate was estimated by the method of Bell and Doisy as modified by Briggs,[4] and chlorid by Volhard-Harvey titration. The Austin and Van Slyke [5] method for plasma chlorid was used in earlier studies; later Van Slyke's [6] new method was substituted. Plasma phosphate was determined at first by Brigg's [4] modification of the Bell and Doisy method and later by the procedure proposed by Benedict and Theis.[7] The other methods used and the general procedure followed in the examination of the blood have already been described.[1]

Treatment during the first few days consisted in giving, in conjunction with insulin, large amounts of fluid and considerable carbohydrate. In the most severe cases a diet was not started until after two or three days of treatment. Carbohydrate was given in the form of 5 per cent. glucose solution subcutaneously and orange juice sweetened with sucrose or lactose by mouth. After the first twenty-four hours milk, cream and lactose were often added in sufficient amounts to approximate a replacement diet. The 5 per cent. glucose solution was given in amounts of 500 c.c. at a time, together with 1,000 c.c. of physiologic sodium chlorid solution once or twice in twenty-four hours. This we believe

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is a valuable aid in increasing the fluid intake, especially in cases with persistent vomiting or coma. Insulin dosage was estimated arbitrarily according to the height of the blood sugar, the persistence of glycosuria and the amount of carbohydrate to be given. It was generally followed within half an hour by a feeding or injection of carbohydrate.

A striking dehydration of the blood has been a prominent feature in all cases of severe diabetic toxemia, and is, we believe, worthy of important consideration in treatment. This dehydration is indicated in the cases shown in Table 1 by the high initial concentration of hemoglobin

TABLE 1.—*Summary of Cases*

Case	Day	Cell Volume, per Cent.	Hemoglobin, per Cent.	Plasma Proteins, per Cent.	Carbon Dioxid, per Cent. by Volume	Blood	
						Sugar, Mg. per Cent.	Nonprotein Nitrogen, Mg. per Cent.
1	Admission	47.2	22.3	7.71	51.1	742	67
	3	45.1	21.3	6.01	76.5	216	39
	31	40.0	18.4	6.44	54.1	297	22
3	Admission	....	20.1	8.24	13.4	616	37
	2	....	20.0	7.66	33.7	344	..
	3	....	16.9	6.14	47.8	349	..
	8	....	16.9	6.44	73.7	249	..
5	Admission	45.8	22.5	8.59	16.8	308	37
	2	42.3	21.0	8.28	41.6	142	..
	3	40.6	19.0	6.41	49.3	229	..
	9	38.1	....	6.05	60.9	241	..
6 First admission	Admission	40.6	18.9	6.95	20.8	309	..
	2	36.7	16.7	6.37	38.3	121	..
	25	24.2	14.8	6.23	66.2	150	..
6 Second admission	Admission	58.2	20.8	7.74	14.5	317	..
	1	51.2	19.5	7.60	19.5	429	..
	2	49.1	20.3	6.90	43.1	278	..
	4	43.8	16.9	6.55	55.6	246	..
7	Admission	43.7	20.1	8.19	43.8	234	..
	3	40.7	17.6	6.87	85.0	113	..
2	Admission	46.2	20.1	7.38	47.8	445	..
	2	46.8	22.2	7.67	49.3	274	..
4	Admission	48.5	20.0	7.43	7.6	450	46
	2	46.2	20.1	7.40	43.0	174	37
	5	37.7	15.4	6.03	51.7	283	29
8	Admission	....	19.8	6.39	22.0	341	37
	2	....	18.3	5.22	50.6	179	24
	10	....	16.2	4.95	64.7	127	24
	17	....	14.8	4.98	61.8	155	24
	27	....	17.5	6.17	67.6	....	23

and of cells in the blood and of protein in the plasma. These changes appear to be due to a decrease in the water of the blood rather than to an absolute increase in the blood elements affected. The cause of this concentration of the blood is not entirely clear. It does not appear to be related to a diminution in fluid intake. Although vomiting was a prominent feature in some cases, concentration was quite as marked in those cases without vomiting. Considerable dilution was effected generally within twenty-four hours and always at the end of forty-eight hours. In the last column of Table 1 are recorded associated changes in plasma carbon dioxid and blood sugar. It seems obvious that the alterations in blood hydration are not related to the level of the blood sugar. Hyper-

glycemia may persist or even increase as the blood becomes diluted. Sherrill and John [8] have shown that the production of hyperglycemia in normal and in diabetic subjects is often accompanied by blood dilution and oliguria. Such reactions and the changes in blood concentration produced by insulin appear to be quite different from those here described.

Haldane and his associates [9] have shown that salts that induce acidosis cause concentration of the blood. Fluid retention resulting from administration of sodium bicarbonate is occasionally met with. The edema associated with diabetes is quickly relieved by the administration of acidotic drugs.[10] Without exception, striking blood dilution during the first two days of treatment was associated with an increase in plasma carbon dioxid, but it must be emphasized that anhydremia may be found when there is little or no acidosis. Patient 7, with marked concentration of the blood, showed only a slight decrease in plasma carbon dioxid. Patients 1 and 2 had both received sodium bicarbonate before admission to the hospital, and neither had much reduction in plasma carbon dioxid. The last case is of considerable interest. The patient had a pneumonia and died shortly after the second blood was taken. It was impossible for her to take large amounts of fluid by mouth. In spite of the fact that glycosuria and acetonuria were relieved by insulin the blood became more concentrated. It is interesting that Patient 1 had slight edema of the ankles and shins though there was definite evidence of blood concentration. Between the first two observations of Case 4 there was a considerable increase of carbon dioxid without any associated change of blood concentration.

In Tables 2 to 8 are recorded the results of analysis of each separate specimen of urine voided during the first few days that the patient was in the hospital, together with the associated changes in the blood and plasma. In several instances the study was continued by analyzing the urine from twenty-four hour periods only. Therapy in each case was instituted as soon as the first specimen was obtained. The top row of figures in each table shows, therefore, the state of the blood and urine before treatment was begun. The volume of each specimen of urine is recorded in the third column. Estimations of the rate of urine excretion, shown in Column 3, were obtained by dividing the volume by the

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TABLE 3.—*Case 3. Third Admission*

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(10)	(11)	(12)	(13)	(14)	(15)	(16)	(18)	(19)
		Urine								Plasma				Blood		
			Volume	Nitrogen			Sodium							Oxygen	Nonprotein	
1923	Time	Volume, C.c.	Hour, C.c.	Gm. per Liter	Gm. per Hour	Gm. per 24 Hours	Chlorid, Gm. per Liter	Glucose, Gm. per Liter	Acetone	Protein, per Cent.	Carbon Dioxid, per Cent. by Volume	Sodium Chlorid, Mg. per Cent.	Inorg. Phosphorus, Mg. per Cent.	Capacity, per Cent. by Volume	Nitrogen, Mg. per Cent.	Sugar, Mg. per Cent.
Nov. 19	10:00 p. m.	225	...	2.83	....	....	0.42	7.6	4+	8.24	13.4	629	5.8	20.1	37	616
Nov. 20	4:30 a. m.	275	42	3.30	0.13	....	0.62	14.3	4+	....	....	...	...	....	..	...
	8:30 a. m.	275	69	5.05	0.35	....	0.90	21.7	3+	....	....	...	...	....	..	...
	10:30 a. m.	...	...	....	....	....	....	....	...	7.66	33.7	602	2.9	20.0	..	344
	4:00 p. m.	350	44	6.69	0.29	....	2.58	19.4	2+	....	....	...	...	....	..	...
	8:00 p. m.	285	82	7.26	0.59	....	2.42	19.5	+	....	....	...	...	....	..	...
	10:00 p. m.	150	75	8.93	0.67	8.0	3.52	18.6	+	....	....	...	...	....	..	...
	12:00 m.	190	95	7.90	0.75	....	3.04	18.2	+	....	....	...	...	....	..	...
Nov. 21	6:00 a. m.	600	100	8.72	0.87	....	5.12	2.2	+	....	....	...	...	....	..	...
	8:00 a. m.	235	117	8.70	1.02	....	0.24	3.8	±	....	....	...	...	....	..	...
	8:30 a. m.	...	...	....	....	....	....	....	...	6.14	47.8	607	1.5	16.9	..	349
	1:30 p. m.	330	60	3.23	0.19	....	1.84	2.7	0	....	....	...	...	....	..	...
	5:00 p. m.	615	185	3.74	0.66	....	2.80	7.6	...	....	....	...	...	....	..	...
	7:00 p. m.	550	275	3.54	0.97	....	2.88	4.6	0	....	....	...	...	....	..	...
	10:00 p. m.	310	103	3.19	0.33	15.1	2.58	0	0	....	....	...	...	....	..	...
Nov. 22	5:00 a. m.	530	76	4.65	0.35	....	3.68	55.8	+	....	....	...	...	....	..	...
	8:30 a. m.	320	72	5.66	0.52	....	2.48	63.2	+	....	....	...	...	....	..	...
	11:30 a. m.	350	117	4.00	0.54	....	2.50	56.1	0	....	....	...	...	....	..	...
	3:00 p. m.	300	57	7.13	0.40	....	4.70	0	0	....	....	...	...	....	..	...
	7:30 p. m.	430	96	4.86	0.46	....	2.84	0	0	....	....	...	...	....	..	...
Nov. 23	1:00 a. m.	430	78	5.05	0.39	10.5	2.60	0	0	....	....	...	...	....	..	...
	6:00 a. m.	250	50	5.37	0.27	....	3.36	0	0	....	....	...	...	....	..	...
	11:15 a. m.	500	95	4.90	0.47	....	....	30.0	0	....	....	...	...	....	..	...
	4:15 p. m.	310	62	6.65	0.41	....	....	0	..	....	....	...	...	....	..	...
	9:00 p. m.	400	97	6.07	0.59	....	....	3.6	+	....	....	...	...	....	..	...
Nov. 24	3:30 a. m.	520	80	4.92	0.39	12.5	....	24.2	3+	....	....	...	...	....	..	...
	5:30 a. m.	400	230	3.10	0.71	....	....	22.2	4+	....	....	...	...	....	..	...

time intervening between that and the preceding voiding. Of course there was no assurance that the bladder was emptied completely each time and the irregularities encountered were to be expected. The nitrogen excreted each hour, shown in Column 6, was estimated in a similar way. Column 7 shows the nitrogen excreted during twenty-four hour periods, starting at the time of admission. One specimen frequently overlapped two twenty-four hour periods. The nitrogen allotted to each was then estimated by interpolation. Columns 8, 9 and 10 record the concentrations of sodium chlorid, inorganic phosphorus and glucose, respectively, in grams per liter, Column 10 the relative intensity of Legal's nitroprussid test for acetone.

In all cases of severe diabetic toxemia the dehydration of the blood was associated with the production of urine containing remarkably low concentrations of nitrogen and sodium chlorid. As the condition of the patients improved and the blood became diluted the concentration of these substances in the urine increased with surprising rapidity. These paradoxic changes occurred in the absence of any constant variation in the rate of urine excretion. Furthermore, there seemed to be no relationship between urine concentration and any individual constituent of the blood. The nitrogen and salt intake was invariably low and could not possibly have accounted for these changes.

In each case of severe diabetic toxemia the concentration of nitrogen in the admission specimen of urine was low. In four instances concentrations as low as 2.5, 2.6, 2.7 and 2.8 gm. per liter, respectively, were encountered. The rate of urine excretion could not be determined in these cases before starting treatment, but the volume output immediately afterward suggests that it was not excessive and probably was relatively low. Apparently oliguria and even anuria may frequently occur in diabetic toxemia. An increase in protein metabolism in this condition is well known and it seems rational to conclude that we are concerned here with a definite failure to eliminate nitrogen. Table 2 shows the study of a case of severe diabetic toxemia. The concentration of nitrogen in the urine (Column 5) was very low on admission but increased steadily and became comparatively normal in less than eighteen hours. The rapid increase in the rate of excretion (Column 6) is even more striking. At a later admission the same patient presented similar phenomena (Table 3). In this one instance the nonprotein nitrogen of the blood and the rate of nitrogen elimination increased simultaneously (Column 18).

In the study of Case 5 (Table 4) the concentration of nitrogen in the urine rose from 6 to 16 gm. per liter in twenty-four hours. Changes of a similar nature are also recorded in Tables 5, 6 and 7. In all of these cases, following the institution of treatment, there was a steady

TABLE 3.—*Case 3, Third Admission*

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(10)	(11)	(12)	(13)	(14)	(18)	(19)
		Urine								Plasma			Blood	
				Nitrogen										
Date, 1924	Time	Volume, C.c.	Volume per Hour, C.c.	Gm. per Liter	Gm. per Hour	Gm. per 24 Hours	Sodium Chlorid, Gm. per Liter	Glucose, Gm. per Liter	Acetone	Protein, per Cent.	Carbon Dioxid, per Cent. by Volume	Sodium Chlorid, Mg. per Cent.	Nonprotein Nitrogen, Mg. per Cent.	Sugar, Mg. per Cent.
Sept. 6	10:30 p. m.	...	...	2.49	....	....	0.16	30.9	4+	....	19.0	...	31	402
Sept. 7	3:00 a. m.	235	52	3.19	0.17	7.3	0.36	28.5	2+	....	....	...	..	...
	10:40 a. m.	775	109	3.63	0.37	....	0.31	24.8	4+	....	....	...	..	...
	8:00 a. m.	...	...	....	....	....	....	....	...	5.06	27.6	590	37	...
	3:30 p. m.	575	120	4.07	0.63	....	2.48	15.0	4+	....	....	...	..	...
Sept. 8	4:30 a. m.	600	46	....	....	....	....	....	...	....	....	...	..	...
	9:30 a. m.	330	66	7.03	0.50	....	5.02	22.5	2+	....	....	...	..	...
	8:00 a. m.	...	...	....	....	....	....	....	...	3.22	47.0	608	37	233
Sept. 8–9	24 hours	2,370	99	5.73	0.56	13.6	4.06	35.0	4+	...	....	...	..	...
Sept. 9–10	24 hours	4,060	169	3.74	0.63	15.2	1.82	32.2	4+	....	....	...	..	...
Sept. 10–11	24 hours	2,245	94	3.56	0.33	8.0	2.01	25.4	4+	4.75	28.0	...	35	257
Sept. 11–12	24 hours	2,215	92	5.27	0.49	11.7	2.24	32.2	+	....	....	...	..	...
Sept. 12–13	24 hours	1,542	64	6.17	0.40	9.5	2.48	11.5	3+	....	....	...	..	...
Sept. 13–14	24 hours	2,910	121	3.79	0.46	11.0	....	18.6	3+	....	....	...	..	...
Sept. 16	8:00 a. m.	...	...	....	....	....	....	....	...	6.70	59.8	...	29	270

TABLE 4.—*Case 5*

(1)	(2)	(3)	(4)	(5)	(6)	(8)	(9)	(10)	(11)	(12)	(13)	(14)	(15)	(16)	(17)	(18)	(19)
		Urine								Plasma				Blood			
				Nitrogen													
Date, 1924	Time	Volume, C.c.	Volume per Hour, C.c.	Gm. per Liter	Gm. per Hour	Sodium Chlorid, Gm. per Liter	Inorg. Phosphorus, Gm. per Liter	Glucose, Gm. per Liter	Acetone	Protein, per Cent.	Carbon Dioxid, per Cent. by Vol.	Sodium Chlorid, Mg. per Cent.	Inorganic Phosphorus, Mg. per Cent.	Oxygen Capacity, per Cent by Vol.	Cell Volume, per Cent. by Vol.	Nonprotein Nitrogen, Mg. per Cent.	Sugar, Mg. per Cent.
Jan. 28	11:40 a. m.	100	..	5.83	....	0.36	0.09	45.8	3+	8.59	16.8	555	4.3	22.5	45.8	37	30.8
	5:20 p. m.	212	35	6.52	0.23	0.64	0.38	51.4	3+	....	....	...	...	....	....	..	...
Jan. 29	8:30 a. m.	430	29	14.68	0.42	3.28	0.31	16.5	+	8.28	41.6	...	2.4	21.0	42.3	..	228
	1:30 p. m.	140	28	15.96	0.45	6.34	0.24	0	0	....	....	...	...	....	....	..	...
	8:35 p. m.	300	43	12.30	0.53	4.82	0.29	23.1	0	....	....	...	...	....	....	..	...
Jan. 30	8:30 a. m.	...	..	....	....	....	....	....	...	6.41	49.5	648	2.9	39.0	40.6	..	...
	11:55 p. m.	600	22	5.83	0.13	3.74	0.49	19.4	0	....	....	...	...	....	....	..	...
Jan. 31	3:00 p. m.	585	39	6.94	0.27	5.82	1.24	21.6	0	....	....	...	...	....	....	..	...

and rapid increase in the nitrogen concentration of the urine until normal or relatively high values were obtained. Almost without exception there was also an increase in the rate of urine excretion during this period resulting in a striking increase in the rate of nitrogen elimination. In some cases the initial rise in the rate of elimination reached an abnormally high point, later declining to a more or less constant level. This is well illustrated in Tables 2, 4 and 6. The maximum rise occurred at the end of about thirty-six hours although normal concentrations were frequently present at the end of eighteen hours. The excessive nitrogen excretion that was frequently found during the second and third days suggests that during the period of anhydremia and dilute urine there is a retention of nitrogen that is swept out as the dehydration is overcome. Several of these cases have not shown much retention of nitrogen in the blood, but in Case 1 (Table 1) on admission there was a definite elevation of nonprotein nitrogen that fell to normal in twenty-four hours. In two other instances, Cases 4 and 8, the blood nonprotein nitrogen diminished as the concentration and elimination of nitrogen in the urine rose.

The alterations of the urinary sodium chlorid concentration were similar to, but more marked than, the nitrogen changes. The dehydration was attended by extremely low concentrations of sodium chlorid in the urine (Column 8 in each table). The admission urine from Patient 3 (Table 3) was practically chlorid-free. With adequate treatment the urinary chlorid concentration rose to many times the original value in less than twenty-four hours. The steady increase and maximum rise corresponded in time to the similar changes of nitrogen. With Case 3 (Table 2) the plasma chlorid decreased somewhat, yet during the same period the chlorid in the urine increased from 0.4 to 6.2 gm. per liter. In only one instance (Table 4) was the increase of chlorid in the urine associated with a great increase of plasma chlorid.

Similar paradoxic changes were encountered in the relationship of blood and urine sugar. This is illustrated by Case 3 (Table 2). The urine at the time of admission contained only 7.6 gm. of glucose per liter while the blood sugar was 616 mg. About twelve hours later the urine sugar had increased to 21.7 gm. per liter while the blood sugar had fallen to 344 mg. Although the blood sugar decreased about one half, the urine sugar increased approximately threefold. Similar but less marked changes in blood and urine sugar were found in Case 6 (Table 6). It is apparently a well known fact that with the approach of diabetic coma the sugar in the urine may disappear.[11] Joslin [12] states,

11. Epstein, A. A., and Felsen, J.: Am. J. M. Sc. **153**:58 (Jan.) 1917.
12. Joslin, E. P.: The Treatment of Diabetes Mellitus, New York, 1923, p. 380.

TABLE 5.—*Case 6*

(1)	(2)	(3)	(4)	(5)	(8)	(9)	(10)	(11)	(12)	(13)	(14)	(15)	(16)	(17)	(19)
		Urine							Plasma				Blood		
Date, 1924	Time	Volume, C.c.	Volume, per Hour, C.c.	Nitrogen, Gm. per Liter	Sodium Chlorid, Gm. per Liter	Inorg. Phosphorus, Gm. per Liter	Glucose, Gm. per Liter	Acetone	Protein, per Cent.	Carbon Dioxid, per Cent. by Volume	Sodium Chlorid, Mg. per Cent.	Inorganic Phosphorus, Mg. per Cent.	Oxygen Capacity, per Cent. by Volume	Cell Volume, per Cent.	Sugar, Mg. per Cent.
Feb. 9	7:00 a. m.	370	...	....	1.18	0.83	....	4+	....	....	...	...	....	....	...
	8:00 a. m.	...	...	....	....	....	....	...	6.95	20.8	553	3.6	18.9	40.6	309
	2:00 p. m.	455	65	5.75	2.02	0.65	41.1	4+	....	....	...	...	....	....	...
	4:30 p. m.	305	122	7.90	3.30	0.72	42.4	4+	....	....	...	...	....	....	...
	10:15 p. m.	215	37	11.24	5.02	0.78	8.7	3+	....	....	...	...	....	....	...
Feb. 10	4:00 a. m.	415	72	5.87	3.14	0.25	2.7	2+	....	....	...	...	....	....	...
	4:30 p. m.	692	56	6.40	5.52	0.35	2.2	+	6.37	58.3	565	5.5	16.7	39.7	121
	6:00 p. m.	300	120	....	....	1.56	....	+	....	....	...	...	....	....	...
	8:00 p. m.	225	112	....	1.68	1.05	....	+	....	....	...	...	....	....	...

TABLE 6.—*Case 6, Second Admission*

(1)	(2)	(3)	(4)	(5)	(6)	(10)	(11)	(12)	(13)	(14)	(15)	(16)	(17)	(19)
		Urine						Plasma				Blood		
				Nitrogen										
Date, 1924	Time	Volume, C.c.	Volume per Hour, C.c.	Gm. per Liter	Gm. per Hour	Glucose, Gm. per Liter	Acetone	Protein, per Cent.	Carbon Dioxid, per Cent. by Volume	Sodium Chlorid, Mg. per Cent.	Inorganic Phosphorus, Mg. per Cent.	Oxygen Capacity, per Cent. by Volume	Cell Volume, per Cent. by Volume	Sugar, Mg. per Cent.
Nov. 10	2:00 p. m.	155	...	2.76	....	34.8	2+	7.74	14.5	530	8.9	20.8	58.2	317
	7:45 p. m.	127	22	2.47	0.055	33.2	...	....	....	...	...	....	....	...
	10:55 p. m	420	140	3.17	0.44	35.0	...	7.60	19.5	505	5.9	19.5	51.2	429
Nov. 11	2:30 a. m.	655	175	4.27	0.74	35.9	...	....	....	...	...	....	....	...
	6:45 a. m.	508	119	5.93	0.71	49.7	...	6.90	43.1	500	3.7	20.3	49.1	278
	11:30 a. m.	366	56	9.34	0.68	54.5	...	....	....	...	...	....	....	...
	3:45 p. m.	125	29	....	....	0	...	....	....	...	...	....	....	...
Nov. 12	5:00 a. m.	150	...	15.52	....	0	...	....	....	...	...	....	....	...
Nov. 12-13	24 hours	1,135	...	11.01	....	43.7	...	....	....	...	...	....	....	...
Nov. 13-14	24 hours	1,285	...	9.98	....	28.0	...	6.55	55.6	568	5.3	16.9	43.8	246

"It is certainly true that one often encounters falling percentages of urinary sugar as coma advances." Epstein, Reiss and Branower [13] have shown that high blood sugar frequently occurs after operation without glycosuria and that at the same time there is a diminution in phenolsulphonephthalein excretion.

The phosphate of the urine in three cases was studied during the initial period of rapid improvement and the observations of other authors confirmed. Quite unlike the nitrogen and chlorid, the urinary phosphate concentration on admission was relatively high and fell rapidly with treatment. In each case this decline was followed by a rise reaching concentrations in two instances much greater than those found in the initial urine specimens. The reduction may be ascribed to the rapid removal of phosphate from the blood in the demobilization of carbohydrate. For the later rise no obvious explanation offers itself.

Fitz [14] has reported studies of the renal function in seven cases of impending or true diabetic coma. High blood urea was frequently found and in all cases the concentration of urea and sugar in the urine was low in comparison with that of the blood. In one fatal case in which the acetone bodies of the blood and of the urine were studied, urinary acetone decreased steadily without any corresponding diminution of blood acetone. Fitz suggests that the acetone bodies may exert a specific toxic effect on the kidneys. He made no determinations that would indicate the changes in the concentration of the blood.

Warburg [15] has reported some cases of "diabetic coma with uremia." Two patients were admitted to the hospital in coma with very high blood sugar and Kussmaul breathing. At first diuresis was slight and very high urea values were found in the blood (129 and 270 mg. per cent). In one case there was no albumin in the urine and in the other a moderate amount. Both patients were restored to health with insulin therapy, the blood urea falling to normal in the course of about two weeks. Later kidney function tests showed no definite abnormalities. He describes another case of a girl, aged 20, with known diabetes, who was admitted in coma. She was treated inadequately with insulin and fluids, and developed anuria and died. No blood urea determinations were made. The kidneys at necropsy were said to present gross evidence of acute parenchymatous nephritis, but no histologic study was made. Warburg remarks that "it does not appear to be generally acknowledged that the kidneys often become strongly deficient in diabetic coma." He has reviewed the history of diabetic coma thoroughly and states that oliguria and anuria are common.

13. Epstein, A. A.; Reiss, Joseph; and Branower, Jacob: J. Biol. Chem. **26**:25 (Aug.) 1916.
14. Fitz, Reginald: Kidney Function in Diabetes, Arch. Int. Med. **20**:809 (Nov.) 1917.
15. Warburg, Erik: Acta med. Scandinav. **59**:301, 1925.

TABLE 7.—*Case 4*

(1)	(2)	(5)	(8)	(10)	(11)	(12)	(13)	(14)	(16)	(17)	(18)	(19)
		Urine				Plasma			Blood			
Date, 1925	Time	Nitrogen, Gm. per Liter	Sodium Chlorid, Gm. per Liter	Glucose	Acetone	Protein, per Cent.	Carbon Dioxid, per Cent. by Volume	Sodium Chlorid, Mg. per Cent.	Oxygen Capacity, per Cent. by Volume	Cell Volume, per Cent. by Volume	Nonprotein Nitrogen, Mg. per Cent.	Sugar, Mg. per Cent.
March 22	5:00 p. m.	2.63	1.55	4+	4+	7.43	7.6	587	20.0	48.5	46	450
March 23	5:00 p. m.	5.34	3.46	±	0	....	....	....	....	....	..	...
March 24	9:00 a. m.	6.88	4.40	±	0	7.49	43.0	601	20.1	46.2	37	174
	4:00 p. m.	6.19	3.87	2+	0	....	....	....	....	....	..	...
March 27	8:30 a. m.	....	....	...	...	6.03	51.7	601	15.4	37.7	29	283

TABLE 8.—*Case 8*

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)	(15)	(16)	(18)	(19)
		Urine									Plasma				Blood		
				Nitrogen										Inorganic			
Date, 1925	Time	Volume, C.c.	Volume per Hour, C.c.	Gm. per Liter	Gm. per Hour	Gm. per 24 Hours	Sodium Chlorid, Gm. per Liter	Inorganic Phosphorus, Gm. per Liter	Glucose	Acetone	Protein, per Cent.	Carbon Dioxid, per Cent. by Volume	Sodium Chlorid, Mg. per Cent.	Phosphorus, Mg. per Cent.	Oxygen Capacity, per Cent. by Volume	Nonprotein Nitrogen, Mg. per Cent.	Sugar, Mg. per Cent.
March 4	6:00 p. m.	385	...	3.24	.....	....	1.50	0.195	1+	2+	6.39	22.0	...	2.4	....	..	...
	11:00 p. m.	1,315	263	2.81	0.740	....	2.72	0.136	1+	3+	....	....	....	....	....	..	...
March 5	5:10 a. m.	1,400	227	2.49	0.566	1.17	2.12	0.158	2+	+	....	....	....	....	....	..	...
	11:30 a. m.	950	150	2.03	0.305	....	2.54	0.112	3+	2+	....	....	....	....	....	..	...
	4:40 p. m.	1,300	263	1.72	0.453	....	2.00	0.072	2+	+	....	....	....	....	....	..	...
	10:35 p. m.	1,370	228	1.57	0.359	....	1.40	0.053	0	0	....	....	....	....	....	..	...
March 6	3:45 a. m.	830	160	1.41	0.226	....	1.26	0.090	+	+	....	....	....	....	....	..	...
	8:30 a. m.	1,030	217	1.41	0.305	....	1.36	0.127	2+	2+	5.22	50.6	578	1.8	18.3	24	179
March 6–7	24 hours	4,575	(190)	1.34	.....	6.14	1.49	.....	...	...	....	....	....	...	....	..	...
March 8–9	24 hours	2,315	(96)	1.95	.....	4.52	1.34	.....	...	...	....	....	....	...	....	..	...
March 9–10	24 hours	2,335	(97)	1.06	.....	2.48	1.75	.....	...	...	....	....	....	...	....	..	...
March 14	8:30 a. m.	.....	...	....	.....	....	....	.....	...	...	4.55	64.7	622	3.3	16.2	24	127

It is impossible to draw any definite conclusions as to whether there is a causal relationship between the concentration of the blood and this inability to concentrate urine. It is interesting to consider the possibility of an increase of osmotic resistance due to an increase in the plasma colloids. The plasma proteins in some of our cases were not extremely high. In all of the cases reported here there were more or less albumin and hyaline and granular casts in the urine before treatment was begun. The albumin and casts in these toxic states suggest local kidney changes. Some disturbance of renal circulation might well explain the functional alterations observed; structural damage to the kidney is hardly conceivable in view of the rapid recovery of function that follows well directed therapy. The excessive concentration of the urine encountered during the second or third days without constant comparable variations in blood constituents suggests that alterations in general tissue metabolism may play some rôle in the phenomena observed.

In this connection it is interesting to contrast Cases 8 and 4. Patient 8 was admitted to the hospital after a long period of malnutrition. He complained chiefly of weakness and marked loss of weight. There had been no recent change in his condition and acidosis had probably been present for some time. His diabetes was moderately severe but his condition was not immediately serious. In this case the dilution of the blood following treatment was quite characteristic but the concentration of nitrogen in the urine decreased steadily, quite in contrast with the other cases reported here. It is also to be noted that this patient had a marked diuresis from the first. The concentration of chlorid during the first twenty-four hours of treatment was somewhat greater than that of the admission urine, but the change was not great. An insufficient diet rather high in proteins probably explains the high nitrogen excretion on admission. The diminishing excretion following treatment was probably due to the fact that increased utilization of carbohydrate and fat permitted the storage of protein to compensate for the preceding period of undernutrition.

Patient 4 also had diabetes of moderate severity but was admitted to the hospital in a very serious condition. He had been careless in following treatment and the day before admission became cold, vomited several times and later developed headache and severe epigastric pains. On admission he was in a state of restless coma. Hyperventilation was extreme. His skin was cold and cyanotic, the temperature subnormal and the pulse weak and rapid. He was obviously very sick. To this condition as contrasted with that of Case 8, we have applied the term "toxemia." The term has not been used as synonymous with either acidosis or ketosis but to distinguish the cases in which the condition

appears immediately grave; cases generally complicated by infection and showing in addition to marked ketosis and dehydration, stupor or coma and various manifestations of circulatory failure. With improvement in his condition this patient did exhibit the characteristic rapid alteration in the concentration of the urine, but a blood examination forty hours after the institution of treatment revealed no change in concentration, although the patient had been given large amounts of fluids and the plasma carbon dioxid had increased 35 per cent. by volume.

There was therefore quite a contrast between the conditions of the two patients, conditions effecting a marked difference in the production of urine. The former showed a rapid dilution of the blood but not the changes in the concentration of the urine which have been characteristic of diabetic toxemia. The latter showed the increasing concentrations of the urine while the concentration of the blood remained unaltered. This suggests that the dilute urine may not be directly dependent on the dehydration of the blood but on some other changes characteristic of the toxemia.

These studies have emphasized certain important considerations in treatment. They not only indicate clearly the importance of giving large amounts of fluids but also the urgent need of combating the toxic destruction of protein. These considerations are all the more important in cases complicated by infection in which there is additional protein destruction, and in cases complicated with kidney disease in which there is a greater tendency for nitrogen retention. It seems clear that glycosuria in itself can be disregarded and large amounts of carbohydrate given in conjunction with insulin, not only to reduce ketosis and acidosis but as a means of sparing the toxic destruction of proteins.

CONCLUSIONS

In severe diabetic toxemia with marked dehydration of the blood the concentration of nitrogen and chlorid in the urine is extremely low. With adequate treatment there is a rapid dilution of the blood and a rapid increase in the concentration of these substances in the urine.

There is no direct relationship between the alterations in the blood and the urine and none of the changes appear to be significantly affected by the presence or degree of hyperglycemia or glycosuria.

The bearing of these phenomena on the treatment of diabetic toxemia has been considered.

REPORT OF CASES

CASE 1.—A woman, aged 47, who was known to have had diabetes for at least a year, entered the hospital with the history of cough and "a cold in her chest" for two weeks and persistent vomiting for four days. There had been nocturia for three or four years and pyuria at intervals. For several days before admis-

sion she had received large doses of sodium bicarbonate. She was well nourished but appeared acutely ill. Respirations were not increased in rate or depth but she was definitely drowsy. The mouth was dry. There was slight cardiac enlargement and scattered râles throughout the lungs. There was slight subcutaneous edema of both shins and ankles. The temperature was 96 F., and the pulse 100 per minute. The systolic blood pressure was 180, and the diastolic 98. The urine showed considerable sugar and acetone and an abundance of pus. The blood nonprotein nitrogen was 67 mg. per hundred cubic centimeters.

The patient entered the hospital in the afternoon of December 11. By the following morning she had received 30 units of insulin but vomiting continued, interfering with the administration of fluid. The blood nonprotein nitrogen was still 67 mg. During the second day the râles in her chest cleared and she seemed to be retaining some fluid. At the time of the second blood examination, about forty hours after admission, she had received 64 units of insulin and was somewhat improved. The edema was slightly more marked. The urine contained no sugar or acetone. Because of the continuous vomiting, the low plasma chlorid and gastric anacidity, she was given 1,000 c.c. of physiologic sodium chlorid solution by hypodermoclysis. Improvement was rapid. Five days after admission the phenolsulphonephthalein excretion was 63 per cent. in two hours and ten minutes. She was finally discharged on a maintenance diet without insulin.

Case 2.—The patient was a woman, aged 57, who had had glycosuria and hypertension for at least three years. Five weeks before admission she developed a cold which gradually grew worse. Two days before admission there was marked respiratory distress with blood tinged sputum. She had been given sodium bicarbonate by mouth. On admission the patient was semicomatose, with rapid shallow respirations and marked cyanosis; obviously she was extremely ill. The temperature was 101 F., the pulse 120 and respirations 42 per minute. The lungs showed evidence of extensive generalized bronchopneumonia. The heart was slightly enlarged and the blood pressure elevated, the systolic being 170 and the diastolic 80. The urine showed considerable sugar, a moderate amount of acetone, a faint trace of albumin and an occasional hyaline cast.

She was admitted at 7 p. m., Dec. 13, 1922. It was very difficult to give anything by mouth, and very little nourishment was administered. By means of small doses of insulin the urine was freed from sugar and acetone by the following morning. Later that day the patient vomited several times, a total of 600 c.c. She became involuntary of urine and feces, grew progressively worse and died after being in the hospital fifty-four hours. The total fluid intake while in the hospital was about 4,000 c.c.

Case 3.—A woman, aged 50, entered the hospital, July 26, 1923, complaining of weakness, thirst and polyuria. The symptoms had begun suddenly about eight weeks earlier. She was slightly emaciated but not extremely ill. There was no hypertension. The urine was negative except for sugar. After three weeks she was discharged on a maintenance diet requiring small doses of insulin.

After discharge from the hospital the improvement in her general condition was not marked. The few weeks before the second admission cooperation and consequently treatment were unsatisfactory. Four days before the second admission she was seized with severe abdominal pains and vomiting. The pain and vomiting persisted. She was moderately emaciated, with dry skin and mucous membranes, breathing deeply with evident air hunger. She was nearly comatose and obviously quite ill. The pulse was rapid but regular. There was some tenderness in the epigastrium but no spasm or rigidity. The weight was 125 pounds (56.7 kg.). The urine showed albumin, a few hyaline and granular casts and large amounts of sugar and acetone. The blood count showed 6,100,000 red blood cells and 13,200 leukocytes. She was admitted at 10 p. m., Nov. 19, 1923. During the first twenty-four hours she received 185

units of insulin, together with fluids containing sucrose, by mouth and 3,100 c.c. of physiologic sodium chlorid solution and 700 c.c. of 5 per cent. glucose by hypodermolysis. At the end of this period there was very little change in her general condition. During the second twenty-four hours she received 85 units of insulin, with continuation of the sucrose by mouth. On the morning of the third day she was definitely improved and her diet was started. She was finally discharged on her previous diet requiring larger doses of insulin.

She entered the hospital a third time, Sept. 6, 1924, with a history similar to that preceding the previous admission except that vomiting and abdominal pain had continued for only twenty-four hours. She was very weak and drowsy with deep respirations and evidence of marked dehydration. The heart was rapid with numerous extrasystoles. The urine was negative except for sugar and acetone. She was admitted about 10:30 p. m. and at once given 1,000 c.c. of physiologic sodium chlorid solution and 500 c.c. of 5 per cent. glucose by hypodermoclysis with 40 units of insulin. Orange juice with sucrose was given in small amounts at a time. During the night she received 40 units more of insulin and the following morning nausea and abdominal pain had lessened. During the day, however, vomiting continued, and she was again given physiologic sodium chlorid solution and 500 c.c. of 5 per cent. glucose under the skin and fluids limited to 50 c.c. every half hour by mouth. By midnight she had received an additional 95 units of insulin and her condition was much improved. She continued to improve clinically. On the morning of September 10 she got out of bed without permission, became dizzy and vomited. Vomiting continued and there was a return of acidosis and air hunger. The plasma carbon dioxid fell to 28 per cent. by volume. The diet was stopped and she was allowed only orange juice and water by mouth, with insulin as required. Under these circumstances she recovered rapidly and the diet was resumed the next day. After one month in the hospital she was discharged on a diet containing 50 gm. of protein, 150 gm. of fat and 100 gm. of carbohydrate, requiring 136 units of insulin daily.

Case 4.—A man, aged 33, was first admitted to the hospital in November, 1923, for the repair of bilateral inguinal hernia. He did not regain his strength after the operation and later developed marked polyuria and began to lose weight and strength. Glycosuria was diagnosed and he again entered the hospital in March, 1924. At this time he was fairly well nourished and had no acidosis. The weight the day of admission was 145 pounds (65.8 kg.). The urine sugar cleared rapidly with insulin and he was discharged on a diet containing 70 gm. of protein, 200 gm. of fat and 100 gm. of carbohydrate, requiring 10 units of insulin daily.

The patient apparently did quite well on this regimen, but about two months before the second admission stopped insulin and dieting. Polydypsia and polyuria then became marked and he began to lose weight. A productive cough developed. The day before admission he became very cold, vomited several times and later developed headache and severe epigastric pain. He could not remember anything that happened the day of admission.

He was admitted, March 22, 1925, at 4:40 p. m. obviously in a very serious condition. He was comatose but restless and thrashed about the bed; he was considerably emaciated. The respirations were very deep and increased in rate. The skin was quite cold with slight general cyanosis; the mouth was extremely dry and the throat was inflamed. The heart was regular and not enlarged, the rate being 84. The lungs showed dulness, bronchial breathing and râles in the right interscapular region. The abdomen was negative, the liver not palpable. The systolic blood pressure was 80, the diastolic 60; the temperature was 96 F. The urine showed considerable sugar and acetone, a moderate amount of albumin and many granular casts. The sputum showed acid-fast bacilli. The weight, eight days after admission, was 133 pounds (60.3 kg.).

Soon after admission he was given 1,500 c.c. of physiologic sodium chlorid solution subcutaneously. When urged he would take fluids by mouth and was given, in addition to water, sweetened orange juice and, later, mixtures of milk, lactose and cream. On the second day he also was given 1,000 c.c. of physiologic sodium chlorid solution and 500 c.c. of 5 per cent. glucose. By the time of the second blood examination, forty hours later, he had received approximately 285 gm. of carbohydrate, 40 gm. of fat and 15 gm. of protein, together with 8,600 c.c. of fluid and 210 units of insulin. Improvement had been marked. Four weeks later he was on a diet containing 75 gm. of protein, 250 gm. of fat and 125 gm. of carbohydrate, requiring 15 units of insulin daily.

Case 5.—A woman, aged 56, was brought to the hospital in coma. Five years before she had had a similar illness, said to have been diabetic coma. Following this she lived on a restricted diet until a few months before admission. During those few months the urine showed varying amounts of sugar but the patient felt well, losing only slightly in weight. Two days before she entered the hospital she became stuporous and up to the time of admission had not been aroused from this condition.

She was completely unconscious. The skin and mucous membranes were quite dry. The pulse was 108 per minute and regular. The respirations were deep. The temperature was 101 F. The heart was moderately enlarged and the peripheral arteries showed definite sclerosis. There were no hypertension and no signs of cardiac failure. Over the base of the right lung there was dulness with diminished breath sounds and râles. Examination of the abdomen revealed nothing of special note. There were no neurologic signs. The urine showed a slight trace of albumin and considerable sugar and acetone. The sediment contained numerous hyaline casts. The blood count showed 6,250,000 red blood cells and 27,000 leukocytes.

The patient was admitted to the hospital at 11 a. m., Jan. 28, 1924. With constant urging she could be made to swallow water and sweetened orange juice. In addition 2,000 c.c. of physiologic sodium chlorid solution and 1,000 c.c. of 5 per cent. glucose were given by hypodermoclysis during the first day, making a total of 5,000 c.c. of fluid. By the time of the second blood examination the following morning, she had received 200 units of insulin. The mental condition was unchanged, but the respirations had quieted down. During the next twenty-four hours she received, in addition to fluids by mouth, physiologic sodium chlorid solution and 500 c.c. of 5 per cent. glucose by hypodermoclysis, together with 145 units of insulin. On the third day she was apparently conscious and responded to stimuli but did not speak and seemed somewhat confused. With further treatment her general condition improved, but aphasia persisted. Three weeks later she developed a pneumonia and at about the same time a pyelitis. These conditions cleared up slowly. She was discharged from the hospital four months later on a diet containing 50 gm. of protein, 150 gm. of fat and 100 gm. of carbohydrate and requiring 45 units of insulin daily. Later insulin was gradually discontinued. The aphasia has persisted to the present time.

Case 6.—A white man, aged 36, whose diabetic symptoms started suddenly ten weeks before admission, following an attack of severe acute epigastric pain, had lost considerable weight and complained of great weakness. The week before admission his general condition was definitely worse, the appetite had failed, marked constipation had developed, and weakness had become extreme. He had been nauseated and just before admission vomited once. He looked quite sick. Except for marked emaciation and definite hyperpnea, physical examination showed nothing important. His mouth was not particularly dry and there was no noticeable stupor. His weight was 103 pounds (46.7 kg.). The systolic blood pressure was 124, the diastolic 86. The admission specimen of urine showed a slight trace of albumin, considerable sugar and acetone and

a negative sediment. The blood showed 5,500,000 red blood cells and 11,200 leukocytes. The blood plasma before treatment revealed a moderate lipemia which had cleared by the next examination.

He was admitted to the hospital on the evening of Feb. 8, 1924, and received nothing but non-nutrient fluids over night. The first blood and urine were obtained the next morning before treatment was started. He was put on a diet at once. During the first five days part of the carbohydrate was given as orange juice. February 9, he was given 50 gm. of protein, 150 gm. of fat and 110 gm. of carbohydrate with 90 units of insulin. The fluid intake was 3,400 c.c. On the following morning there was evidence of definite improvement. February 10, he received 50 gm. of protein, 150 gm. of fat and 145 gm. of carbohydrate with 35 units of insulin. The fluid intake was 3,500 c.c. By the end of this period there had been considerable improvement. He was discharged at the end of one month aglycosuric, on a diet of 50 gm. of protein, 200 gm. of fat and 100 gm. of carbohydrate, without insulin.

Following discharge from the hospital the patient did fairly well. After a few days insulin had to be resumed. At first from 10 to 20 units each week was sufficient but after several months his requirement had gradually increased to 40 units daily. The diet had remained constant except for occasional indiscretions. Later it appears that he became somewhat careless about his diet and four days before the second admission stopped taking insulin. The night before admission there was a sudden onset of nausea and vomiting followed by intense abdominal pain. Vomiting continued up to the time of admission. He was emaciated and greatly dehydrated. Respirations were almost maximum in depth. He was somewhat stuporous and not clear mentally. The mucous membranes were slightly cyanotic. The pulse was rapid and thready. The systolic blood pressure was 112, the diastolic 85; the temperature was 99 F. The abdomen was held tense but nowhere showed definite spasm. The admission specimen of urine contained considerable sugar and acetone, a large amount of albumin and many coarse and fine granular casts. Examination of the blood showed 5,600,000 red blood cells and 30,000 leukocytes. The condition seemed very grave and the abdominal symptoms were so marked that surgical operation was seriously considered.

He was admitted to the hospital the second time at 1:45 p. m., November 10. Soon after admission he was given 1,000 c.c. of physiologic sodium chlorid solution and 500 c.c. of 5 per cent. glucose by hypodermoclysis, with 40 units of insulin. Four hours later the abdominal pain had subsided and vomiting stopped. For the next four hours he was given 50 c.c. of orange juice every half hour with a total of 60 units more of insulin. From then until 5 p. m., November 11, he received 25 c.c. of orange juice, 25 c.c. of water and 2.5 gm. of lactose every half hour with an additional 160 units of insulin. Twelve hours after admission he showed considerable improvement; hyperpnea had practically disappeared. November 12, a diet containing 50 gm. of protein, 150 gm. of fat and 100 gm. of carbohydrate was started. He was discharged from the hospital on the twenty-fifth day requiring 60 units of insulin daily.

Case 7.—A Jew, aged 37, entered the hospital with a history of polyuria, polydypsia, weakness and loss of weight during the previous three years. There had been a recent marked exacerbation of symptoms with a cold and double otitis media. He was of unusually small stature, extremely emaciated and markedly dehydrated. On admission both ear drums had ruptured and there was a marked rhinitis. The respirations seemed somewhat increased but the plasma carbon dioxid was normal. The urine contained a trace of albumin and a large amount of sugar and acetone; microscopic examination was negative. A diet containing 50 gm. of protein, 100 gm. of fat and 50 gm. of carbohydrate was started at once. Improvement was rapid. During the two days between the first and the second blood examinations, he received 150 units of insulin and

(though not advised by the attending staff) 40 gm. of sodium bicarbonate. He was discharged later on a diet containing 50 gm. of protein, 175 gm. of fat and 100 gm. of carbohydrate with 45 units of insulin daily.

CASE 8.—A man, aged 28, was admitted to the hospital after about four months of diabetic symptoms. These symptoms had developed suddenly about three weeks after an acute upper respiratory tract infection. Polyuria and polydypsia were marked, and he had lost considerable weight and strength. There had been no particular change in the general condition during the few weeks before admission. There was a marked family history of obesity but the patient was a very small man, his average weight being from 110 to 115 pounds (49.9 to 52.1 kg.). On admission he was extremely emaciated, weighing only 65 pounds (29.5 kg.). He was mentally clear and not drowsy. There was no cyanosis. The mouth was somewhat dry. The respirations were slightly but definitely increased in depth. The lungs were clear and the heart negative. The liver edge was palpable 1 inch (2.5 cm.) below the costal margin. The urine showed a very slight trace of albumin, considerable sugar and acetone and nothing important in the sediment.

He started at once to take a diet containing 50 gm. of protein, 150 gm. of fat and 100 gm. of carbohydrate. During the first twenty-four hours he received in addition 600 c.c. of orange juice and 130 units of insulin. Rapid improvement followed and he was discharged four weeks later on a diet containing 60 gm. of protein, 175 gm. of fat and 110 gm. of carbohydrate, requiring 25 units of insulin daily.

THE DIAMETER OF THE RED BLOOD CELLS IN THE DIFFERENTIATION OF ANEMIAS

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Advances in the treatment of Addison's anemia in the last few years are so full of promise that it is now of more importance than ever to make an early and decided diagnosis of this disease. If the full benefits of the newer treatment [1] are to be obtained, the diagnosis should be made at least early in the first period of anemia. Indeed, in some cases it is possible to make the diagnosis so early, even before the anemia is apparent, that it may not be in vain to hope that at times the disease as we now know it may be entirely prevented.

It is to aid in the more exact differentiation of the anemias that this work is presented, for it is believed that careful measurement of the red blood cells helps materially in such differentiation. This thesis was presented originally by Price-Jones in England,[2] was later enlarged on by Shackle and Hampson,[3] and more recently by Hurst [4] and his co-workers. To the present time, however, little has appeared in the American literature on the subject, and no "red cell diameter curves" have been published. Capps [5] in 1903 measured the diameter in a number of diseases, but his notes on technic are incomplete, and he drew up none of the curves later devised by Price-Jones.

In this paper is described a simplified technic for the measurement of red cells and for the construction of the curves; curves are shown from normal subjects, from patients with Addison's anemia, from patients with secondary anemia, and from patients with a few other diseases, and the conclusions that one is forced to adopt from this work are indicated.

The ingenious method for recording the diameters of red cells devised by Price-Jones is shown by the curve in Figure 1. The solid line is a reconstruction much reduced of the normal curve recorded by Price-Jones on the measurement of 10,000 red cells in twenty nor-

1. Hurst, A. F.: Medical Essays and Addresses, New York, Paul B. Hoeber, 1924. Vanderhoof, D.: The Etiologic Relation of Achylia Gastrica to Combined Sclerosis of the Spinal Cord, Arch. Int. Med. **32**:958 (Dec.) 1923.
2. Price-Jones, C.: The Diameter of Red Cells in Pernicious Anemia and in Anemia Following Hemorrhage, J. Path. & Bacteriol. **25**:487 (Oct.) 1922.
3. Shackle, J. W., and Hampson, A. C.: Megalocytic and Nonmegalocytic Anemia, Guy's Hosp. Rep. **74**:193 (April) 1924.
4. Hurst (Footnote 1).
5. Capps, J. A.: A Study of Volume Index, J. M. Res. **10**:367, 1903-1904.

mal subjects. The ordinates show the diameter of the cells in microns; the abscissa, the number of cells of each different diameter. At a glance one can see the largest and the smallest cells encountered in a spread (i. e., the degree of anisocytosis), the frequency with which a particular size occurs, and the position of the peak of the curve, which corresponds rather accurately with the average diameter.

TECHNIC

The technic for measuring the diameter of the cells as described by Price-Jones and as modified by Shackle and Hampson is exceedingly difficult to accomplish. It consists essentially of projecting the microscopic field either by means of a camera lucida or a projecting microscope to a screen, on which the image of the cell is either measured directly or its outline drawn and the outline measured. Two diameters are measured on each cell, the average taken, and a curve drawn from the average diameters of 500 cells. Such a procedure is so laborious and time consuming that it is hardly practical for use in clinical medicine.

The procedure used in this work was devised before we knew the technic of the English workers. Our measurements were made by means of a Leitz ocular micrometer. This is a glass disk, on which is engraved an arbitrary scale of fifty divisions, which fits into the ocular of the microscope so that the image of the scale focuses on the image of the blood film on the microscope stage. The scale is first calibrated for the particular set of lenses and tube length to be used, by means of an object micrometer, a slide on which are engraved lines a known distance apart.

When the value in microns of the divisions of the scale of the ocular micrometer is known, one is ready to proceed with the measurements. A blood film is brought into focus. Two or three cells lying on the scale are measured to fifths of a space. The slide is moved a short distance by the mechanical stage, and the two or three cells that happen to lie on it again are measured. As the measurements are made they are called off and an assistant marks off a space for each cell in the proper column on cross-ruled paper. In this way a curve is built up as the count proceeds. When 200 cells are measured the final curve is drawn by connecting the heads of the columns, and then a scale is drawn in showing the value of each column in microns. To complete the curve the numerical average diameter of the cells is determined.

The foregoing procedure may be criticized in a number of respects. We have no doubt sacrificed accuracy for practicability to some extent, but our results agree so closely with the results of the English workers

that we feel that this procedure, by which a red cell diameter curve can be made with about the same expenditure of time and labor as a differential white count, warrants consideration.

It will be noted that we measure only one diameter in each cell instead of averaging two. This necessitates selecting round cells for measurement, for it is obvious that oval cells would give incorrect values. No doubt selecting just the round cells causes certain inaccuracy, but the error probably works in both directions and tends to neutralize itself.

Even averaging two diameters does not, as Capps points out, give a perfect picture of the size of the red cells, which are, of course, three dimentional bodies. The thickness of the disks no doubt varies and may be of some clinical significance. Capps feels that the volume of

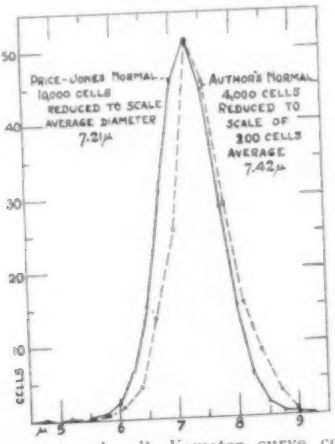

Fig. 1.—Author's normal red cell diameter curve compared with that of Price-Jones.

the red blood cells can be measured accurately by his method of estimating the "index volume." In the strict sense our curves are not volume curves but diameter curves. This does not, however, invalidate the conclusions that may be drawn from the red cell curves or their significance in differential diagnosis.

Our method of sampling is possibly less accurate than if we measured all the cells in a given field. One could, of course, make the curves entirely inaccurate if one measured only the large cells or only the small cells, but successive counts on the same slide give curves that are so similar that we feel a reasonably accurate sample is obtained if one moves quickly across a slide and counts all of those round cells that happen to fall on the micrometer scale.

Two hundred cells do not, of course, give as representative a sample as the 500 that the English workers use. Price-Jones [6] has estimated that according to statistical laws an error of 0.2 microns is to be expected if 500 cells are counted, 0.32 microns if 200 cells are counted. In practice our variations have not been this much. In measuring two sets of 200 cells each on the same slide, our mean diameters have not been more than 0.3 microns apart, a difference that we feel is not of enough importance to warrant measuring more cells. Measuring 500 cells is a difficult task even with the simplest technic. It cannot be accomplished at one sitting and tends to make the procedure impracticable for clinical use. Two hundred cells can, however, be measured in from ten to fifteen minutes after a little experience.

The best argument for the foregoing procedure is the fact that our curves agree so closely with those of the English workers. In Figure 1 and all succeeding figures, the broken line represents our normal curve obtained from the measurement of about 4,000 cells in nineteen normal subjects, reduced to 200 cells. It will be noted that the contour of the curve corresponds quite closely with that of Price-Jones. The two limbs of our curve, however, are to the right of the limbs of the Price-Jones curve, which makes our average normal diameter 7.42 microns instead of 7.21 microns.

This brings up one of the most important and difficult parts of the procedure no matter what technic is used, i. e., calibrating the micrometer scale. Whether one measures the cells directly by means of an ocular micrometer or whether one measures the diameter of the projected image by means of a millimeter rule, one must first calibrate the scale used with an object micrometer in order to determine the value of the divisions of the scale in microns. One cannot depend on the theoretical magnification of a certain set of lenses at a certain tube length and reduce the reading by this magnification.

We calibrated our micrometer with a Zeiss Objektmikrometer on which are engraved lines 0.01 mm. apart, with two counting chambers on which the double ruled lines are 25 microns apart, and with a slide engraved by Queen and Company in 0.001 inch. These slides gave slightly different readings. It is obvious that only one reading can be correct, but as a matter of fact some variation was found on different parts of each slide. The Zeiss Objektmikrometer, for instance, at one point showed five spaces of the ocular micrometer (which is about the diameter of the average red cell) equal to 7.58 microns, at another point equal to 7.15 microns. A Leitz blood counting chamber at one point gave five spaces in the ocular equal to 7.16 microns, at another

6. Price-Jones, C.: The Diurnal Variations in the Size of Red Blood Cells, J. Path. & Bacteriol. **23**:371 (Dec.) 1920.

7.36 microns. In order to overcome this variation ten readings were taken on each instrument and the results averaged. We appreciate the fact that this does not correct this important source of error entirely, but we know of no more accurate method. When we first calibrated

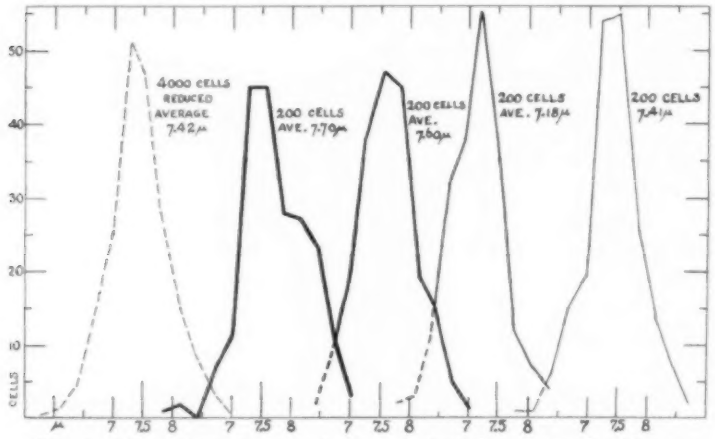

Fig. 2.—More extreme types of curve obtained from normal subjects.

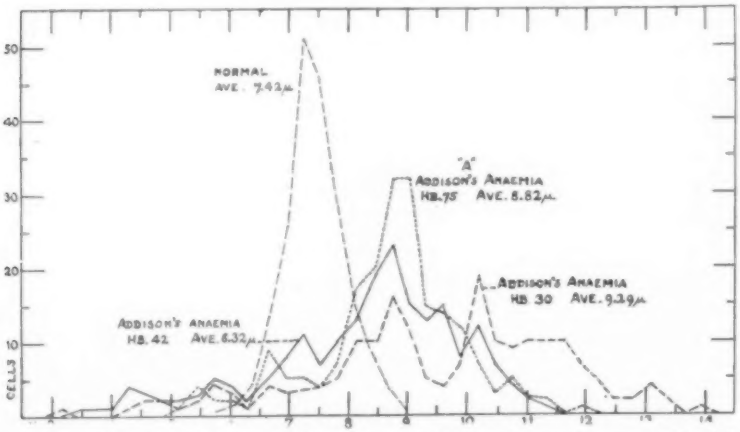

Fig. 3.—Three typical curves from cases of Addison's anemia compared with the normal; the great width of the curves, their irregular character, and that they lie largely to the right of the normal, making the average diameter larger than normal, should be noted.

our ocular micrometer the average of all measurements showed five spaces to equal 7.245 microns. On finding that the average diameter of the red cells in our normal spreads was consistently larger than that of

Price-Jones, we repeated this calibration carefully and obtained practically identical results (7.252 microns). We have no doubt that the English workers appreciated the importance of accurate calibration and that their work was carefully done, but we record our curves as obtained.

In this work a Zeiss No. 4, 10 × ocular was used with a Zeiss 1/12 oil immersion objective, N.A. 1.25, tube length 160 mm. We measured a few spreads by increasing the tube length until one space in the ocular micrometer equaled 1 micron. This would simplify to some extent the making of the curves, but the difficulty is to find the tube length at which one space equals 1 micron. With the instruments at hand we did not feel satisfied with this procedure, and abandoned it in favor of the procedure given above.

NORMAL CURVE

As noted above, we have determined the average normal diameter as 7.42 microns; Price-Jones determined it at 7.21 microns; Shackle and Hampson as 7.23 microns, and Capps as 7.65 microns. Our normal spreads were unselected as to age, sex and time of day. Price-Jones [6] showed a very definite, athough irregular diurnal variation in the average diameter (6), amounting at times to as much as 0.6 microns. Since in clinical medicine, however, spreads are ordinarily made at any time of day, we used the same procedure in this work.

Of more importance than the average diameter in a large group are the extreme types that may be regarded as normal. For this reason Figure 2 is given showing the variations encountered in normal spreads. It will be noted that the average diameters vary from 7.18 microns to 7.70 microns. These are simply the largest and smallest diameters found in our normals, and no doubt slightly larger and slightly smaller diameters will be found in normal persons on further search. But the character of the curve is to be noted as well as the position of the peak. The greatest difference between the largest and the smallest cell in any spread is 3.2 microns, i. e., there is little anisocytosis; the rise to the peak is abrupt, and the fall also, and the peak is well defined and occurs about at the average diameter. These are the characteristics we shall look for in examining the subsequent curves.

ADDISON'S ANEMIA

The most striking variation from the normal occurs, as one might expect in Addison's anemia (Fig. 3). Here there is a wide curve extending over 7 to 11 microns due to the great anisocytosis. There is no well defined peak, but occasionally two or three peaks occur, while the whole curve is pushed well to the right, i. e., the average diameter is increased. These characteristics occur in Addison's anemia regardless

of the stage of the disease. Figure 4 shows two curves from a case during remission. The curve more nearly normal was made when the hemoglobin was 90 per cent. and the patient looked and felt perfectly well. At the same time there is considerable anisocytosis and the curve is pushed well to the right of the normal, making the average diameter 8.6 microns instead of 7.42 microns.

It is probably in the early differentiation of Addison's anemia from secondary anemia that this procedure will be of greatest value. Curve A in Figure 3 illustrates this point. This is from a woman of 62 years who complained of weakness, shortness of breath on exertion, and loss of appetite. If any pallor had developed recently, it was obscured by the fact that she had always been fair and had had white hair for years. Examination showed her well overweight, with a moderate arteriosclero-

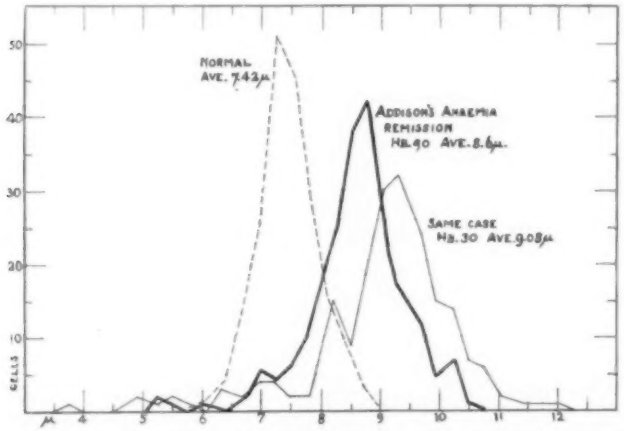

Fig. 4.—Addison's anemia; in remissions the curve approaches normal, but still remains to the right of it.

ris, hypertension, and cardiac enlargement. Her hemoglobin was 75 per cent., and spreads on ordinary examination looked practically normal. Nothing else of any consequence was found and we were about to make a diagnosis as indicated above, which would have explained the clinical picture satisfactorily, but decided to wait for the red cell curve. This showed a very striking variation in the size of the red cells, much more than one would expect from merely looking at the spreads, with the average diameter increased to 8.70 microns. Subsequently, complete absence of hydrochloric acid was found in the gastric juice, completing the evidence that this was undoubtedly an early case of Addison's anemia.

SECONDARY ANEMIA

In secondary anemia, whether due to infection, hemorrhage or new growth, the curves, while not so striking, are characteristic. The degree of anisocytosis is never so great as in Addison's anemia, and the average diameter is always either within normal limits or smaller than

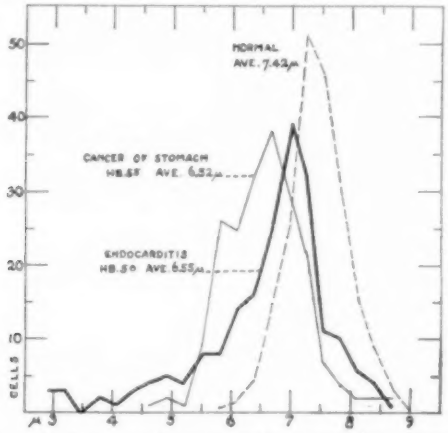

Fig. 5.—Two typical curves from secondary anemia; curves are pushed to left of normal, making average diameter smaller than normal.

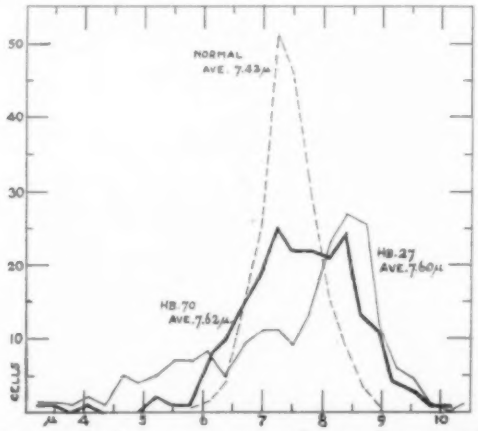

Fig. 6.—Two curves from same case of secondary anemia; while the hemoglobin in the one is very low the average diameter is within normal limits.

normal. Two typical examples are shown in Figure 5. It is important to note that the decrease in the average diameter is not proportional to the decrease in the hemoglobin. A very severe anemia may show red

cells of normal or nearly normal diameter, but the important point is that the average diameter is never larger than the normal and never approaches even the smallest average diameter found in Addison's anemia.

In this connection the most puzzling spreads which we encountered are those shown in Figure 6. They are from a man aged 46, who was brought to the hospital in coma. A history was obtained of at least two periods of severe anemia with fever in the last three years, and of loss of blood from hemorrhoids for the last four to six years. His hemoglobin on admission was 27 per cent.; red cells totaled 1,224,000; white cells, 2,600. A temperature of from 100 to 103 degrees continued from three to four days, gradually subsiding. Later a test meal showed no hydrochloric acid in the gastric juice. His

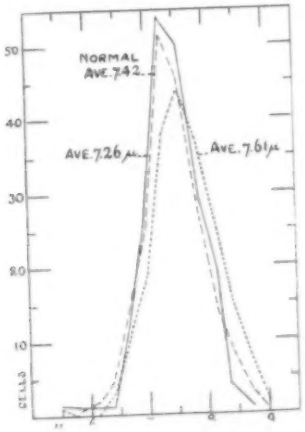

Fig. 7.—Curves from two cases of posterior lateral sclerosis with achylia but without anemia. These curves are essentially normal.

spreads showed considerable anisocytosis and poikilocytosis and the red cells looked about normal in color.

Here was conflicting evidence, the previous periods of anemia with fever, the achlorhydria, leukopenia and superficial examinations of the blood spreads indicating Addison's anemia, the history of prolonged hemorrhage indicating secondary anemia. Measurement of the red cells on several occasions, both while the anemia was at its worst and after the hemoglobin had been brought up to 70 per cent., showed the average diameters of the red cells to be 7.6 microns as shown in Figure 5. As this is about the upper limit of normal, it still remained a question whether this was a secondary anemia with a large normal diameter or an Addison's anemia with a diameter approaching normal. Were it

an Addison's anemia, however, there seems no doubt that at least when the hemoglobin was down to 27 per cent. the average diameter would have been very definitely above normal and the degree of anisocytosis would have been much greater than it was. Whether the achlorhydria played any part in the production of the anemia is not entirely certain, but with the foregoing evidence it seems reasonable to suppose that the anemia was not of the Addisonian type but was secondary to the prolonged hemorrhage.

POSTERIOR LATERAL SCLEROSIS

Curves from two cases of posterior lateral sclerosis without anemia are shown in Figure 7 because of the close association of the disease with Addison's anemia. One of these patients had been under treatment for two years and was practically symptom free when the spreads were made. Both cases had an achlorhydria. The diameter curves are essentially normal. It will be interesting to see whether these patients develop any anemia in the future and whether the curves change.

CONCLUSIONS

A technic has been described for the measurement of the diameter of red blood cells and for the construction of diameter curves which is sufficiently accurate for clinical purposes and sufficiently simple to be used in a routine manner in examining any questionable blood spread.

The diameter curves are of value in the differentiation of Addison's anemia in all its stages and of secondary anemia.

RENAL FUNCTION IN PERSONS HAVING ONLY ONE KIDNEY*

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Man is born with at least twice as much kidney tissue as he requires. This fact is proved by the health of numerous persons who have sustained unilateral nephrectomy. Probably this wide margin of safety in the matter of surplus kidney substance has intimate relation to the difficulty of diagnosis of renal disease in its earlier or milder stages. So far as meeting the necessary demands for excretion it would seem to be an indifferent matter whether one kidney be removed or half the elements of each kidney be destroyed by disease.

For the purpose of the present study we are ignoring certain important features of nephritis, namely, evidence that the disease is constitutional rather than confined to an organ; that its tendency seems progressive; that connective tissue growth in healing within an organ produces of itself an extension of destruction of organic elements. But in the early stages of nephritis, at some time before symptoms of any kind are manifested, diagnosis depends to a considerable extent (though not exclusively) on signs of congestion in the kidney (albuminuria, casts, blood, etc.) or on evidence of impaired renal function. And it is because of the equivocal significance of albumin and casts in the urine that the modern tendency is to attach greater weight to tests of function.

For example, in the case of a man of 40, whose first intimation of unsound health comes as a rejection for life insurance on account of albuminuria, it is often very difficult to determine that albuminuria is due to nephritis, yet the probabilities are that it is.[1] The usual functional tests in such cases are normal and yield no data for diagnosis. The explanation for a normal response to tests in cases ultimately proved to be nephritis in after years may be that the extent of disease does not transgress the margin of safety, which is to say that the amount of normal tissue is adequate to meet the demands of our tests.

It has been suggested by Addis that some of the deficiencies in methods of diagnosis may be overcome by making use of functional tests that impose more strain than is the case with the usual tests. In

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*From the first division, New York Hospital, and Cornell University Medical College.

1. Insurable persons having a faint trace of albumin at the age of 40 or over have a death rate from nephritis which is ten times the normal; with a trace of albumin the death rate is about thirty times the normal; with large amounts of albumin and casts the rate is about fifty times the normal. Dublin, L. S.: Am. J. Hygiene **1**:301 (May) 1921.

principle, I think this idea is sound. And it is certainly true that in many cases of quite definite nephritis the response to such functional tests as phenolsulphonephthalein excretion, the concentration tests of Mosenthal and of Christian, the concentration of urea in the urine or in the blood, are all within the accepted zones of normal. The majority of these cases, however, fail to attain normal standards when tests that do impose considerable strain are used. Tests that come within the last category are the water excretion test of Volhard [2] and the concentration test as modified by Addis.[3] The defect in the water excretion test is due to the fact that renal function depends on the general circulation, hence on the integrity of the heart; but in the class of case in which the test is of most use, namely, in apparently healthy persons, this imperfection is not of serious moment. It is moreover supplemented by the concentration test. In the work of routine diagnosis these two tests have been found of great value. The experience afforded by more than 200 "early" cases suggests that while neither test alone is infallible the results of both together accord well with the later progress of the case.

With the restrictions and assumptions already mentioned, the question then arises as to what extent of injury of renal elements must occur before functional limitation can be detected. Specifically, is it possible to impose sufficient strain to disclose a limitation of functional response when the person has only one kidney, i. e., half the normal amount of functioning elements?

In selecting cases for test we have used only younger persons, since in any one over 40 there is the possibility of degenerations incident to age or vascular disease. These tests have in all cases been conducted within three weeks after the removal of the diseased kidney. This was done in order to exclude the possibility of the effect of hypertrophy of the remaining kidney, although it is generally believed that hypertrophy occurs only when one kidney is removed in early life. All of the cases reported had been studied with great care before operation and in none was there evidence of disease in the kidney that was left in situ. These tests included catheterization of the ureters and examination of the urine from each kidney, response to dye tests, etc. But since the most meticulous study cannot exclude tuberculosis of the kidney, the two cases of nontuberculous disease are most important (Cases 2 and 7). The blood chemistry and phenolsulphonephthalein excretion were normal in all these cases after operation.

First we shall consider the response to the concentration test.[3] During this test the patient was allowed no liquid for twenty-four

2. Volhard: Verhandl. d. 27 Cong. f. inn. Med., 1910. Volhard and Fahr: Die Brightsche Nierenkrankheit, Berlin, 1914.

3. Addis, T., and Drury, D. R.: J. Biol. Chem. **55**:105 (Feb.) 1923.

hours, no water, milk, soup, tea or coffee. He received the usual full "house" diet with these exceptions. The test commenced after breakfast on the first day and continued till breakfast the second day. Each voiding of urine was collected and tested for specific gravity.[4] Especially significant were urines passed during the night and early morning at the end of the period. During the latter hours of this test the majority of normal persons void urine of a specific gravity of 1.030 or 1.034. With a small minority the concentration does not go higher than 1.024 or 1.026. In this series of patients with one kidney, only one responded to this test in a way that is undoubtedly normal (Case 7). The kidney was removed in this instance on account of an infection following an injury. The highest specific gravity in Cases 2 and 8 was 1.028, which may be normal.

Summary of Tests of Renal Function

Case	Diagnosis	Age	Highest Specific Gravity of Urine During Thirst Day	Volume of Urine in Four Hours After Ingestion of 1,500 C.c. of Water	Urea in One Hour's Urine = N / Urea in 100 C.c. Blood: First Hour	Second Hour	Third Hour	Average
1	Tuberculous kidney	14	1.024	1,135	25.1	25.9	36.1	29
2	Carcinoma	32	1.028	1,260	45	28.7	36.4	36.7
3	Tuberculous kidney	29	1.022	945	28.5	29.7	25.1	27.7
4	Tuberculous kidney	21	1.018	840	5.5	1.5	6.5	4.5
4	Same case 5 months later	..	.....	.....	32.4	36.00	34.00	34.1
5	Tuberculous kidney	31	1.026	1,745	36.21	22.87	27.81	28.89
6	Tuberculous kidney	27	1.026	1,426	49.89	29.48	30.08	36.48
7	Pus in kidney	27	1.030	1,600 (1.001)*	11.10	27.50	.....	19
8	Tuberculous kidney	14	1.028	1,330 (1.001)*	20.76	16.08	22.09	19.8
9	Tuberculous kidney	31	1.027	1,014 (1.001)*	4.8	18.72	12.65	12.05
9	Same case 2 weeks later	..	.....	.....	24.24	43.25	40.04	35.8

* Specific gravity.

The water excretion test [2] is conducted as follows: In the morning before breakfast the patient is given 1,500 c.c. of water which he drinks in the course of half an hour. The bladder is emptied before the test and all of the urine is collected thereafter for four hours. Normally more than a liter is excreted during this interval. In only two cases (Cases 3 and 4) was the result below a normal standard. It is perhaps surprising that the average is not lower in the series since it would seem that if in any relation the amount of tissue influences the result it would be apparent in this test. And in cases suspected of nephritis on account of albuminuria this test more often than any other one test is apt to indicate defective function.

4. The concentration of urea was estimated in many cases also; it was normal or above in all. On the concentration of urea especially McLean relies for diagnosis.

By studying the effects of changes in blood-urea concentration on the rate of urea excretion, Addis and Drury came to the conclusion that for special conditions the ratio $\frac{\text{Urea in one hour's urine}}{\text{Urea in 100 c.c. of blood}}$ is a constant for a wide range of blood-urea concentration.

In applying this principle to clinical study two essential conditions are required: (1) The kidney should be placed under circumstances that call for great activity in urea excretion; (2) there should be an absence of certain specific renal stimulants and depressants. The tests in this series have been done in the same manner as described by Addis.[5] Since in our series the urea of the blood was normal, 30 gm. of urea was the dose given in a liter of water. The hourly specimens of urine and the samples of blood were taken with care and analyzed for the urea content. Addis found the ratio in normal adults to average 50.4. Ratios between 34 and 40 are within a zone of doubtful interpretation, but ratios below 34 are abnormal. It is at once noted in our series that in no case does the function of one kidney as expressed in the ratio reach the level of average normal, namely 50. But if the average weight of the single kidney in these cases is 150 gm. the theoretical ratio would be 25. This figure is considerably exceeded in four cases (Case 2, 36; Case 4, 34; Case 6, 36; Case 9, 35). In Cases 4 and 9 the ratio was determined again after an interval and the second result is of interest since it would indicate an enormous increase in the functional ability of the kidney. Two possible explanations suggest themselves for this improvement: the kidney might have hypertrophied, though this is not supposed to occur in adults; or at the time of the first observation the kidney might still have been affected by toxic depression—disease of one kidney is believed to induce this in the other. In both patients there had been a decided improvement in general health at the time of the second observation; possibly this was a factor. But the explanation for the fact must be left to conjecture.

When results of the tests of each patient are considered, not separately but together, as a whole from which some sort of general impression should be gained, the impression is, in two cases at least (Cases 2 and 6), that even these tests which impose severe strain have failed to indicate that the reserves of renal function have been fully determined, or, in other words, the decrease in renal tissue in a person with one kidney is only doubtfully revealed by these tests when the findings are compared with those of a normal subject. And the only test that shows consistently that there is a limitation of function is the ratio after giving urea.

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5. Addis, T.: Renal Function and Amount of Functioning Tissue, Arch. Int. Med. **30**:378-385 (Sept.) 1922.

If we are to depend to any extent on tests of function for the detection of renal disease (and at present there is no alternative) then it seems important to know whether any test can so tax the maximum effort of the organ that a loss of half its functioning tissue will be evident. This question is vital to methods of diagnosis as applied to the less advanced stages of renal degeneration.

In the somewhat artificial conditions imposed by this study it has been necessary to ignore considerations that are possibly of great importance clinically. While some diseased kidneys appear to be a result of discrete areas of degeneration, the neighboring structures remaining healthy, this is not invariably the case. It may happen that all portions of diseased kidney appear abnormal, the differences in various areas being in the degree of departure from the normal. Now it is impossible to predict or even to surmise to what extent even the most trivial change in a cell group will alter the function of these cells. And while the results of study of persons with only one kidney emphasize the limitations of diagnostic methods in the recognition of nephritis they also suggest that caution must be exercised in endeavors to estimate the amount of tissue still capable of function.

HYPERGLYCEMIA

I. THE RELATIVE BLOOD VOLUMES IN DIABETES MELLITUS *

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It has been known for a long time that diabetes mellitus is a disease characterized by a greater severity in young people than in persons at or beyond middle age. In young patients thirst, excessive diuresis and loss of weight are prominent symptoms, standing in sharp contrast to the relative or absolute comfort enjoyed by older people. Exsiccation, acidosis and coma occur frequently, while in older patients they are usually secondary to some definitely assignable cause, such as acute dietary indiscretion, the onset of an acute infection or cerebral hemorrhage. In an elderly patient it is usually only after some similar mismanagement or accident that the disease runs an acute course.

Polyuria and exsiccation are always associated with the severe form of the disease, but they are either inconspicuous symptoms or are absent altogether in the milder forms. It would seem then that diuresis and renal permeability must exert an important influence in determining the course. If so, all cases of severe diabetes, regardless of age, should show evidences of tissue and blood dehydration. All cases of diabetes in young persons should show more evidence of tissue and blood dehydration than occurs in the elderly. Elderly patients should be expected to exhibit scarcely any evidence of dehydration, perhaps none at all in the absence of polyuria. I have tried to show that these suppositions are correct.

The presence or absence of tissue exsiccation has been estimated by clinical observations of the mucous membranes, skin and subcutaneous tissues. Anhydremia and hydremia have been determined by measuring the relative volumes occupied by serum and by erythrocytes in serial samples of defibrinated blood. This has been done by means of the electrical conductivity measurements of the whole blood and of its serum. From these the relative volumes were calculated by means of the relation developed by Stewart.[1] All conductivity measurements have been corrected to 5 degrees C., and are reported as specific conductivities, that is, $K \times 10^4$ at 5 degrees C. The erythrocyte counts were all made in duplicate, with the same pipet and counting chamber. All counts that

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1. Stewart, G. N.: The Relative Volume or Weight of Corpuscles and Plasma in Blood, J. Physiol. **24**:356 (July) 1899.

showed a variation of more than 250,000 were discarded. In each instance the average of the two counts was used for the final figure. These were all made on defibrinated blood. Blood sugar determinations were made by the method of Folin and Wu.[2]

It must be understood that all statements pertaining to the relative blood volumes refer to conditions existing in the circulating blood of an

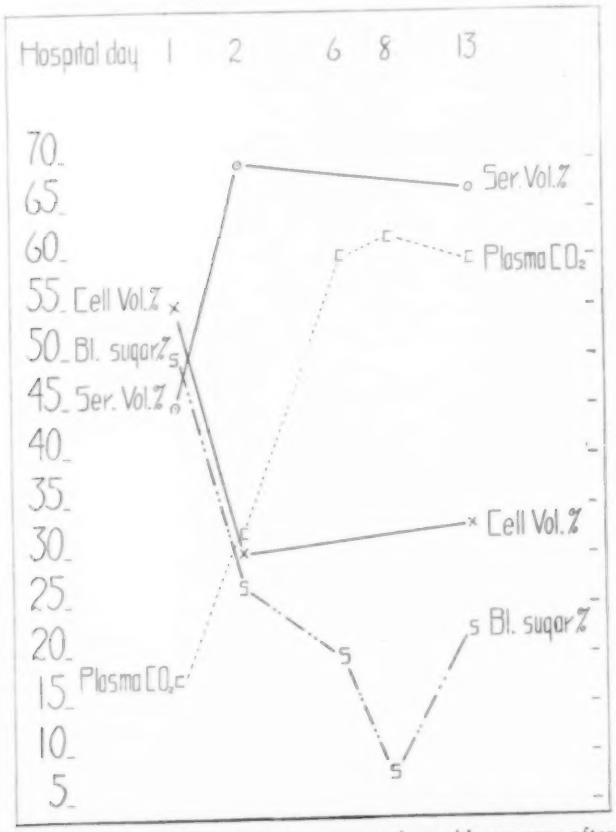

Fig. 1 (Case 33).—Diabetic coma in young patient with recovery after insulin treatment; severe degree of blood concentration on day of admission; effects of insulin: increase in relative volume of serum, increase in plasma carbon dioxid and decrease in blood sugar.

antecubital vein. The greatest possible care was exercised both in collecting and in defibrinating the samples.

2. Folin, O., and Wu, H.: System of Blood Analysis, J. Biol. Chem. **38**:81 (May) 1919.

CHANGES IN YOUNG PATIENTS

In young patients not under treatment it has been found that hyperglycemia invariably produces an increased concentration of the blood. The initial blood sample, taken on admission to the hospital, has always been more concentrated than any succeeding sample. Whenever erythrocyte counts have been made, they have always been higher in the first sample. Unless the patient is anemic, the relative volume of serum has always been less than the normal in the first sample, increasing toward the normal as the patient becomes more and more under the influence of dietetic management and insulin treatment. Then, in some

TABLE 1.—*Blood Concentration Changes in Young Diabetic Patients*

Case	Date	K × 10⁴ at 5 Degrees C.		C.c. Serum in 100 C.c. Whole Blood	Changes in Serum, Percentage by Volume	Blood Sugar, per Cent.	Red Blood Cells in Millions, per C.Mm.	Remarks
		Blood	Serum					
3	1/11/24	30.0	77.2	55.96	.....	0.33	.....	
	1/13/24	32.0	78.2	58.11	+ 2.15	0.15	.....	
5	2/ 6/24	24.6	72.4	50.75	.....	0.37	.....	
	2/11/24	26.2	73.7	52.54	+ 1.79	0.24	.....	
	3/ 7/24	40.1	72.3	74.27	+23.52	0.23	.....	
	3/11/24	32.6	73.0	63.15	+12.40	0.16	.....	
8	3/ 3/24	26.3	74.6	52.06	.....	0.30	.....	
	3/18/24	28.0	74.1	55.19	+ 3.13	0.19	.....	
9	2/ 6/24	24.8	69.4	53.31	.....	0.37	.....	
	2/ 9/24	27.4	76.5	52.51	— 0.80	0.30	.....	
12	3/10/24	27.5	74.4	54.15	.....	0.21	.....	
	3/12/24	28.2	73.2	56.56	+ 2.41	0.33	.....	
16	9/28/23	22.3	65.6	51.66	.....	0.30	.....	
17	10/14/23	26.3	73.0	53.28	.....	0.34	.....	
	6/18/24	25.6	70.4	53.94	— 0.66	0.46	5.840	
20	5/23/24	26.5	73.0	53.61	.....	0.43	5.350	Coma; death
23	6/ 8/24	30.7	76.1	57.88	.....	0.20	4.800	
	6/13/24	33.4	73.2	64.24	+ 6.36	0.18	3.900	
	6/17/24	33.5	73.2	64.22	+ 6.34	0.38	4.300	
28	6/10/24	30.0	74.5	57.98	.....	0.24	5.210	
	6/17/24	32.7	74.5	62.03	+ 4.05	0.22	4.840	
33	9/11/24	19.3	66.7	44.67	.....	0.30	6.450	Coma
	9/12/24	37.8	74.0	69.46	+24.79	0.28	3.545	Recovery
	9/23/24	34.8	72.5	66.80	+22.13	0.23	5.155	
34	9/23/24	50.6	72.8	84.77	.....	0.08	2.445	Secondary anemia
	9/26/24	48.5	70.8	85.97	+ 1.20	0.38	2.855	Coma; death
36	11/20/24	21.1	76.6	42.2	.....	0.38	7.310	Coma; death

cases, the gross viscosity of the blood is visibly decreased. These changes are well shown in the data from the cases presented in Table 1 and also in Figure 1.

Excessive diuresis was present in each case. Every patient was markedly underweight and, despite increased thirst and fluid intake, presented dry mucous membranes and a harsh, dry skin.

CHANGES IN ARTERIOSCLEROTIC PATIENTS WITH ACUTE EXACERBATIONS OF DIABETES

The data in Table 2 represent the same findings in all arteriosclerotic diabetic patients who were admitted to the hospital because of

acute dietary indiscretions, acute infections or cerebral hemorrhage or thrombosis. Here again, the conditions on admission indicated increased blood concentration. If recovery occurred under treatment the blood concentration diminished, while if treatment was unsuccessful and the patient died, the blood became even more concentrated. Figure 2 illustrates a recovery.

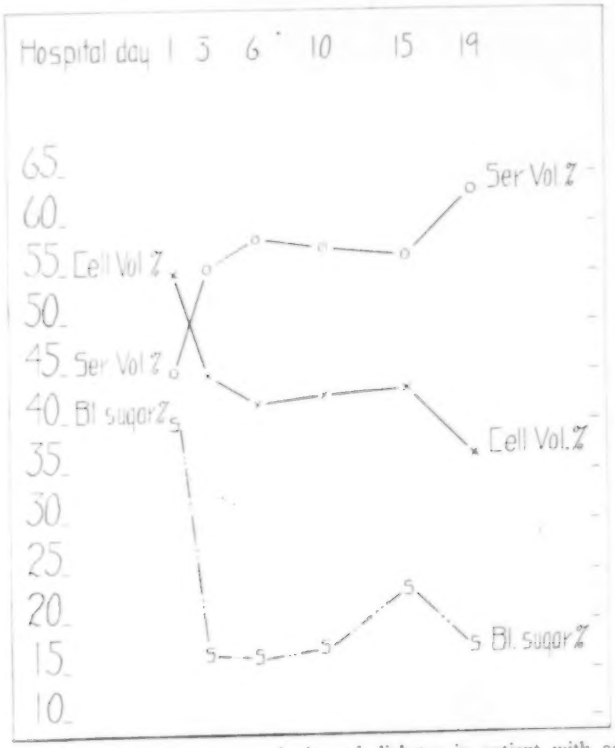

Fig. 2 (Case 15).—Acute exacerbation of diabetes in patient with advanced arteriosclerosis; extreme reduction of relative volume of serum on first day and prompt increase following insulin administration.

Here, also, increased diuresis was a prominent symptom. Emaciation was not observed but in every instance the mucous membranes and skin showed marked evidences of tissue dehydration.

CHANGES IN ARTERIOSCLEROTIC DIABETIC PATIENTS

In this group, represented by the data in Table 3, the initial blood samples do not show any evidence of increased blood concentration. On the contrary the majority of them indicate a blood dilution, and

in the remainder the water content of the blood appears not to have been disturbed. In these patients the response to dietary and insulin treatment is a return from the initial dilution back toward the normal condition, an *increase* in blood concentration (Fig. 3). In some instances the gross viscosity of the blood is visibly increased after the hyperglycemia has disappeared.

These patients did not suffer from thirst and polyuria, and there was only slight clinical evidence of tissue dehydration. In each case examination of the accessible peripheral arteries and of the ocular fundi gave unmistakable evidence of advanced arteriosclerosis. The latter also is true of the cases in Table 2.

TABLE 2.—*Blood Concentration Changes in Arteriosclerotic Diabetic Patients Presenting Acute Exacerbations*

Case	Date	K × 10⁴ at 5 Degrees C.		C.c. Serum in 100 C.c. Whole Blood	Changes in Serum, Percentage by Volume	Blood Sugar, per Cent.	Red Blood Cells in Millions, per C.Mm.	Remarks
		Blood	Serum					
2	12/14/23	23.1	72.1	48.35	.....	0.60	.....	
	12/15/23	34.4	81.6	58.84	+10.49	0.20	.....	Coma
	1/13/24	35.2	74.4	65.67	+17.32	0.12	.....	Recovery
6	2/22/24	32.2	72.0	63.41	.....	0.56	.....	Gangrene of foot
	3/ 7/24	38.5	71.0	73.47	+10.06	0.37	.....	
	3/11/24	40.3	73.0	73.0	+ 9.59	0.51	.....	
11	2/ 3/24	26.0	67.2	57.28	.....	0.52	.....	Coma
	3/ 6/24	23.8	67.5	52.96	— 4.32	0.27	.....	Death
14	4/20/24	26.6	71.9	54.53	.....	0.35	5.330	
	4/21/24	34.5	76.9	62.58	+ 8.05	....	4.215	Coma
	4/22/24	28.6	74.3	55.88	+ 1.35	0.60	5.355	Death
15	5/ 5/24	21.6	73.5	44.67	.....	0.40	7.350	
	5/ 7/24	31.0	80.1	55.48	+10.81	0.17	5.810	Cerebral thrombosis
	5/10/24	31.9	77.2	58.70	+14.03	0.16	5.570	
	5/14/24	32.4	80.0	57.40	+12.73	0.17	5.130	Coma
	5/19/24	30.0	76.1	56.84	+12.17	0.24	5.700	Recovery
	5/23/24	37.9	81.4	63.83	+19.16	0.17	5.920	
19	12/ 9/23	26.4	75.9	51.34	.....	0.25	.....	
21	5/20/24	23.9	73.9	48.56	.....	0.37	6.420	Food indiscretion
	6/11/24	26.3	73.2	52.45	+ 3.89	0.20	5.450	
	6/18/24	28.3	74.8	56.09	+ 6.53	0.18	5.970	
1	11/23/23	34.4	74.5	64.38	.....	0.53	4.700	Gangrene of foot
	11/26/23	41.3	77.2	70.93	+ 6.55	0.15	.....	Acute cellulitis
	12/15/23	43.7	79.1	71.98	+ 7.60	0.14	3.800	Secondary anemia

That actual increase or decrease in the total number of erythrocytes was not a factor in this dilution is shown by the fact that when water balance had been restored, and the bloods remained at equilibrium so far as the relative blood volumes were concerned, the erythrocyte counts and the cell volume percentages corresponded to the normal average ratio found for all treated diabetic patients, whether young or old. This is 5,000,000 erythrocytes per cubic millimeter for 38 per cent. relative cell volume.

CHANGES IN DIABETIC PATIENTS LONG UNDER INSULIN TREATMENT

When diabetic patients have been under constant dietary and insulin treatment for six months or longer, departures from the normal blood

concentrations depend entirely on the blood sugar level at the time at which samples are taken. If the food intake and insulin dosage are well balanced, so that the blood sugar does not rise much above 0.15 per cent., excessive thirst and diuresis are absent and the relative blood

TABLE 3.—*Blood Concentration Changes in Arteriosclerotic Diabetic Patients*

Case	Date	K × 10⁴ at 5 Degrees C. Blood	K × 10⁴ at 5 Degrees C. Serum	C.c. Serum in 100 C.c. Whole Blood	Changes in Serum, Percentage by Volume	Blood Sugar, per Cent.	Red Blood Cells in Millions, per C.Mm.	Remarks
4	1/11/24	32.8	78.2	59.25	.....	0.35	.....	
	1/13/24	32.8	78.1	59.30	+0.07	0.30	.....	
	1/23/24	33.2	77.8	60.08	+0.78	0.16	.....	
7	2/22/24	41.4	77.1	71.21	.....	0.37	.....	
	3/ 8/24	38.6	77.1	67.78	−3.43	0.15	.....	
	3/13/24	33.5	74.4	63.23	−7.98	0.20	.....	
10a	3/ 3/24	31.5	76.0	59.05	.....	0.46	.....	First admission
	3/ 7/24	26.6	73.0	53.71	−5.34	0.21	.....	
	3/11/24	24.8	72.6	50.28	−8.77	0.19	.....	
10b	4/30/24	30.6	76.8	57.09	−1.96	0.33	5.565	Readmission
	5/10/24	31.7	78.8	57.22	−1.83	0.18	5.700	
15	4/18/24	32.6	75.7	60.81	.....	0.30	4.995	
	4/22/24	31.7	76.1	59.19	−1.62	0.27	6.195	Terminal pneumonia
	4/30/24	33.0	75.0	62.0	+1.19	0.17	5.030	
	5/10/24	29.3	77.9	54.40	−6.41	0.26	5.920	Death
22	6/ 8/24	37.5	80.5	63.02	.....	0.07	5.440	
	6/13/24	36.9	75.6	60.37	−3.35	0.15	4.650	
	6/17/24	33.0	76.1	61.11	−2.51	0.21	4.980	
26	6/10/24	29.5	74.8	56.95	.....	0.17	6.740	
	6/17/24	27.5	71.5	56.25	−0.67	0.22	6.080	

TABLE 4.—*Blood Concentration Changes in Diabetic Patients Long Under Treatment*

Case	Date	K × 10⁴ at 5 Degrees C. Blood	K × 10⁴ at 5 Degrees C. Serum	C.c. Serum in 100 C.c. Whole Blood	Changes in Serum, Percentage by Volume	Blood Sugar, per Cent.	Red Blood Cells in Millions, per C.Mm.	Remarks
18	12/ 9/23	29.7	78.0	54.83	.....	0.14	.....	No arteriosclerosis
24	6/10/24	30.7	72.2	60.92	.....	0.14	6.730	
	6/17/24	32.2	73.5	61.99	+1.07	0.12	5.450	Child
25	6/10/24	32.3	74.8	61.09	.....	0.13	6.170	No arteriosclerosis
	6/18/24	33.2	74.8	62.52	+1.43	0.16	5.020	
29	6/10/24	31.4	74.5	60.17	.....	0.16	5.190	Slight arteriosclerosis
	6/17/24	33.2	74.9	62.44	+2.27	0.14	4.840	
30	6/11/24	29.4	72.2	58.86	.....	0.26	5.300	No arteriosclerosis
	6/18/24	28.9	72.2	58.77	−0.09	0.24	4.720	
37	11/20/24	28.8	77.1	54.18	.....	0.21	6.160	Young adult
		28.9	76.7	54.70	+0.52	0.23	5.560	
		27.9	75.4	54.19	+0.01	0.21	5.740	
38	12/ 4/24	29.4	73.6	57.68	.....	0.25	5.210	Young adult
		32.6	75.0	61.41	+3.73	0.25	5.710	
		29.6	72.8	58.76	+1.08	0.30	5.220	
		29.0	74.7	56.36	−1.27	0.23	5.950	
39	2/ 4/25	30.0	71.4	60.5	.....	0.27	4.955	Young adult
		29.5	70.9	60.1	−0.38	0.26	4.630	
		28.6	70.0	59.6	−0.90	0.21	4.700	
		29.2	71.4	59.4	−1.10	0.23	4.485	

volumes are within normal limits. In Table 4, Cases 24, 25 and 29 show the results in this well balanced state.

If there is a departure from the normal blood concentration, the direction of the variation seems to depend on the presence or absence

of arteriosclerosis; with normal arteries there is an increased concentration, whereas in the presence of arteriosclerosis a blood dilution occurs. In other words, during periods of unsatisfactory management there is a return from the satisfactory condition mentioned in the foregoing to that which classified the patient on admission. Cases 30, 37, 38 and 39 in the same table represent young diabetic patients in whom treatment was not satisfactory at the time. In each instance the blood is

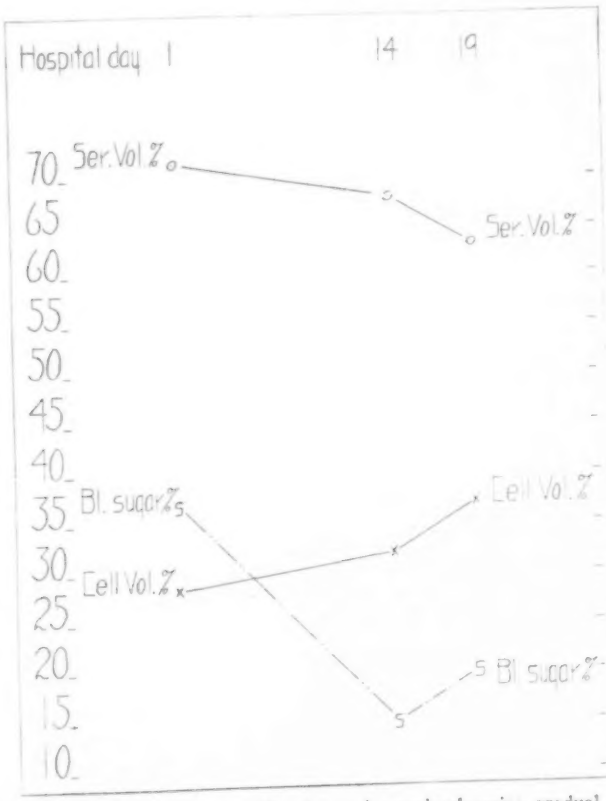

Fig. 3 (Case 7).—Diabetes with advanced arteriosclerosis; gradual onset of symptoms; initial blood dilution; in this case insulin treatment caused a blood concentration.

more concentrated than the average normal. Case 10 in Table 3 shows what happened to an arteriosclerotic diabetic patient during her absence from the hospital. She became careless about her diet, developed a hyperglycemia and again diluted her blood almost to the same extent found on her first admission.

In the event of mismanagement or infection all young diabetic patients will be reclassified into Group 1, and the amount of increased blood concentration will depend on the degree of hyperglycemia that results and the period of time during which the higher level is attained; the more rapid the increment in blood sugar the greater the increase in blood concentration. In the case of arteriosclerotic diabetic patients, if the increment in blood sugar is rapid they will be reclassified into Group 2; but if it is slow, they will fall into Group 3.

Thus the findings in this series of cases of diabetic patients support the hypothesis presented in the second paragraph, and suggest the following explanation: An increase in the concentration of glucose in the blood above the average upper normal limit causes water to be drawn into the blood stream, producing a state of hydremia. If the patient has normal kidneys and blood vessels, glycosuria and polyuria result and lead to tissue exsiccation, then to relative anhydremia or blood concentration, and eventually to severe water loss and acidosis. On the other hand, if there is much arteriosclerosis, even though the blood sugar slowly increases to 0.30 per cent. or 0.35 per cent., only a little glycosuria or polyuria occurs or even none at all, and the result is simply a relative hydremia or dilution of the blood. In young people the increase in fluid intake cannot keep pace with the increased output, but in arteriosclerotic diabetic patients, even though there may be some generalized tissue exsiccation, the fluid intake is sufficient to prevent blood dehydration. However, as I have already shown, if the rise in blood sugar occurs rapidly, all patients, regardless of age or arteriosclerosis, speedily become exsiccated.

CONCLUSIONS

Diabetes mellitus produces marked changes in water distribution and in the total water balance of the body.

The degree of these changes is modified by the magnitude of the hyperglycemia and by the rate of onset.

Their character and direction is modified by vascular disease and renal permeability.

Reduction of hyperglycemia (or the action of insulin) may produce either an increase or a decrease in the concentration and viscosity of the blood. The result in any given case depends on the antecedent water content.

Book Reviews

OPOTHERAPIE ENDOCRINIENNE. LES BASES PHYSIOLOGIQUES, LES SYNDROMES, LA POSOLOGIE DE L'OPOTHERAPIE PAR LES GLANDES A SECRETIONS INTERNES. By GUY LAROCHE, Medecin des Hopitaux de Paris. Pp. 256; illustrated. Paris: Masson et Cie, 1925.

This volume deals with the general principles of organotherapy. It contains one chapter on the chemistry and the processes of production of extracts of animal tissues; one long chapter on the thyroid and thyroid disorders and their treatment; a brief chapter on parathyroids and the thymus; an exhaustive chapter on suprarenal disorders, and shorter chapters on the hypophysis, the testes and ovarian disorders and their treatment. The chapter on the pancreas deals almost exclusively with insulin, and there is a short but sane chapter on the organotherapy of so-called pluriglandular syndromes.

The volume as a whole gives evidence that the author is familiar with the important literature in this field, although reference to this literature is meager. The author further shows good judgment in most cases when dealing with the analysis and conclusions in fields in which great uncertainty still exists both as to etiology and therapy. For example, in the chapter on the so-called pluriglandular disorders and their treatment, he wisely rejects the claim that it is good therapy to give a mixture of glandular extracts because the body will pick out what it needs for its rehabilitation and reject the others. The book may be read with profit by physicians.

OPOTHERAPIE CLINIQUE ORGANOTHERAPIE-FORMULAIRE. By MARCEL LAEMMER. Pp. 151; brief index. Paris: Masson et Cie, 1925.

This volume is essentially a book of formulas of organotherapeutic preparations as used and endorsed by the author, and possibly by other physicians, in many types of disorders. The author lists organotherapeutic products not only from the recognized endocrine glands but also from organs and tissues like the pancreas, the liver, the salivary glands, the intestines, the stomach, the cerebrum, the heart, the lungs, the bones, the muscles, the placenta, the bone marrow, the lymph glands and the mammary glands. All of these, according to the author, have been used with therapeutic success in human disease.

He says, "It is sufficient to have employed with therapeutic success extracts of the heart, the cerebrum or the lung to establish their real therapeutic value." "Such clinical facts exist and let us accept them with joy, even though the way in which these results are obtained still remain obscure." But so far as the reliable clinical literature today is concerned, the success of such therapies is achieved on the principle of post hoc reasoning.

On Page 36 the author states as proved facts that the pancreatic secretion inhibits suprarenal activity; that the thyroid hormone inhibits the pancreas; that the thymic hormone inhibits suprarenal activity, and that the mammary hormone inhibits the ovaries. Without much fundamental discussion the author, on Page 72, gives several formulas of alleged organotherapeutics of value in the treatment of epilepsy. One of these runs: dessicated thyroid, dessicated parathyroid, dessicated ovary, with calcium carbonate.

On Page 102 the following formula is given for the treatment of constipation: dessicated duodenal mucosa, dessicated ox bile, and powdered agar-agar. For chronic nephritis and uremia he gives a mixture of dessicated thyroid, dessicated liver and dessicated heart (Page 117).

On Page 123 we are told that one may use successfully thyroid extract, suprarenal extract, ovarian extract and parathyroid extract for middle ear suppuration. On Page 132 the following formula is given for the treatment of inoperable cancer: dessicated thyroid, dessicated bile, dessicated liver, bone marrow, with manganese sulphate.

The volume seems totally unreliable as a guide for physicians. The book might be understandable as a satire on the credulities and follies of ancient and modern organotherapy, but taken as a serious contribution to biology and medicine, one would have to look far for a monograph on organotherapy equally credulous and unreliable.

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